

Supplementary Information

Association of extensive RNA disruption with natural killer cell-mediated death of K562 chronic myelogenous leukemia cells

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*

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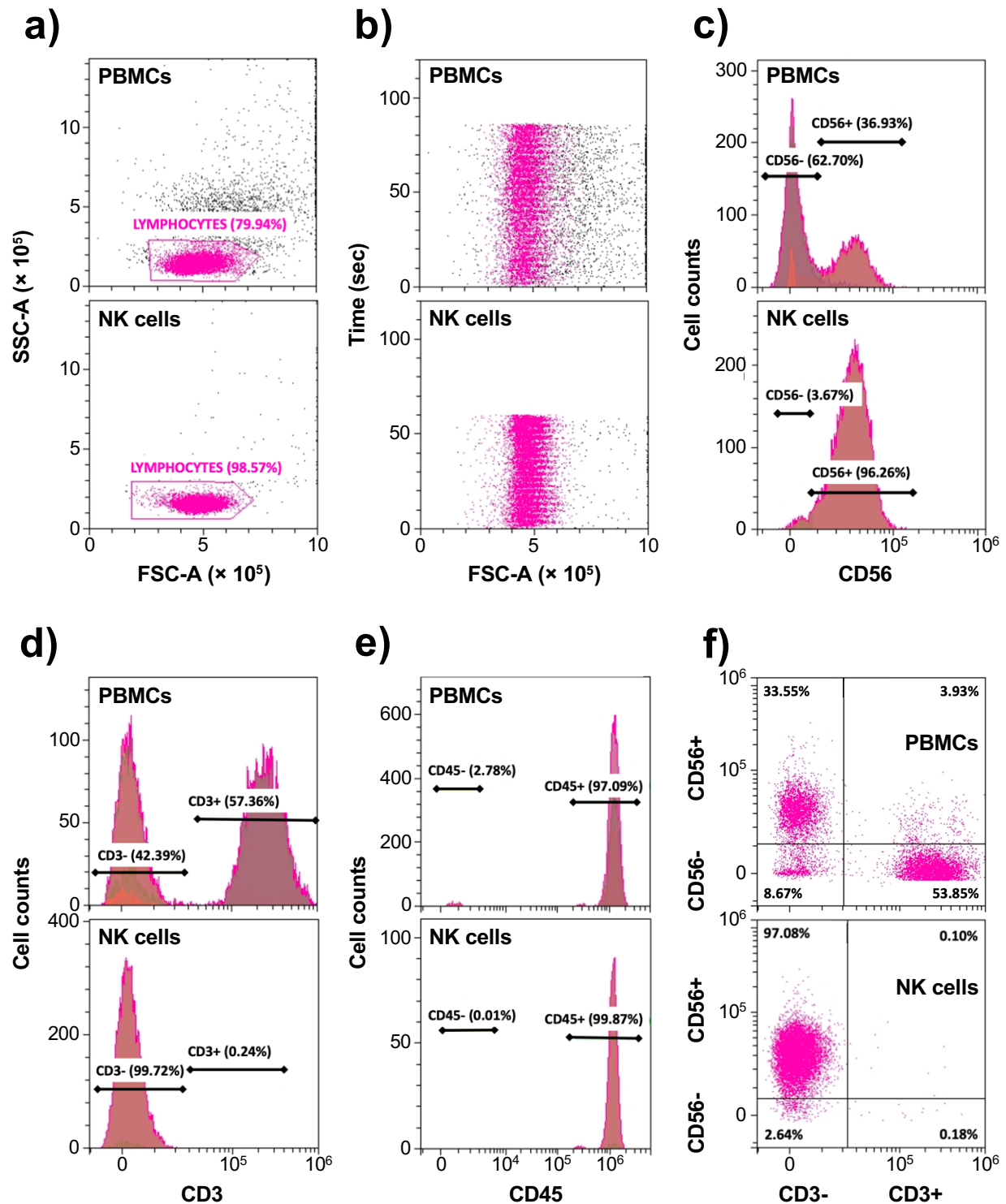
³ Rna Diagnostics, Inc., Sudbury and Toronto, ON, Canada

⁴ Division of Medical Sciences, Northern Ontario School of Medicine, Sudbury, ON, Canada

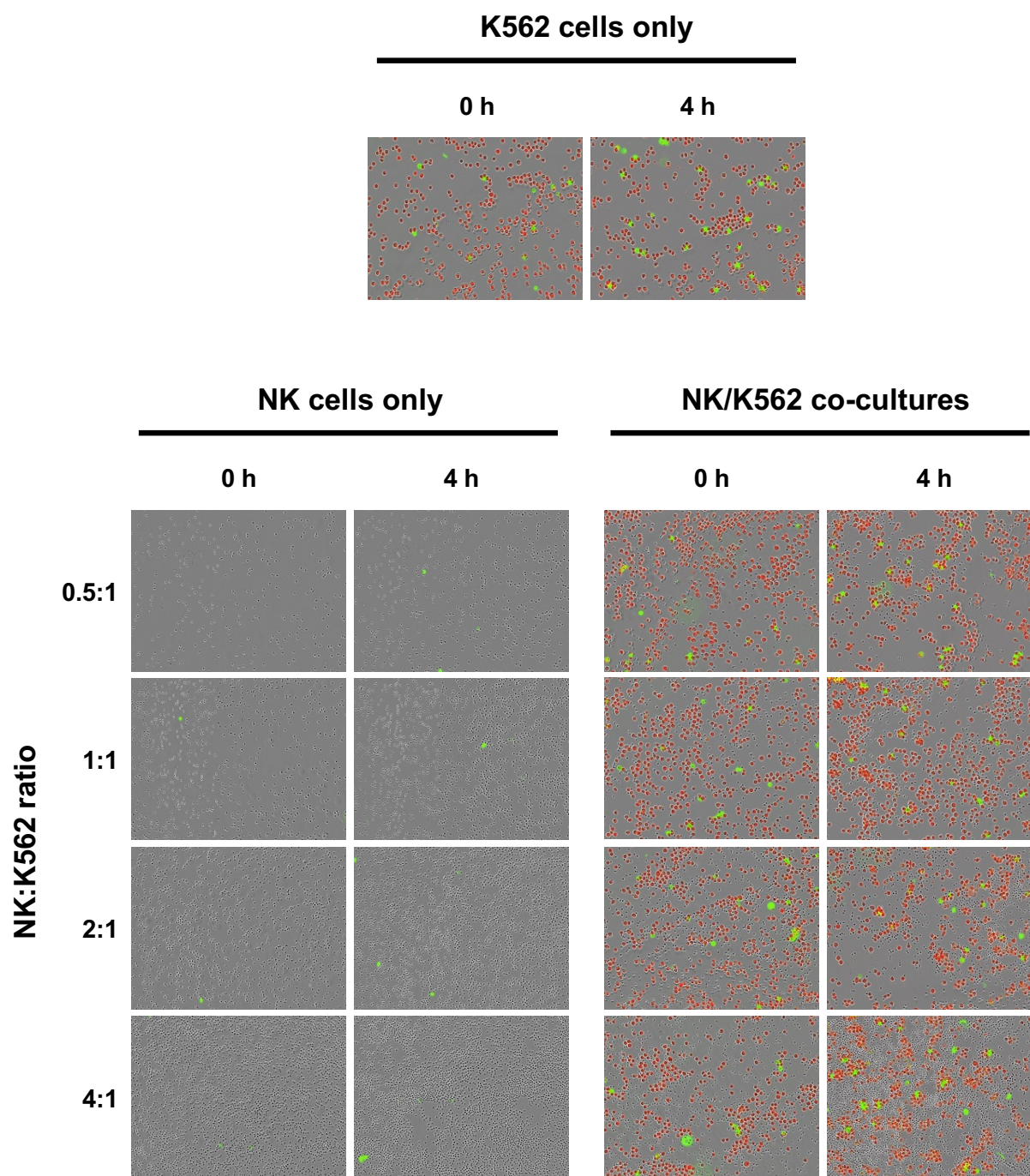
***Corresponding author:** Amadeo M. Parissenti, telephone: 1-705-523-7300 ext. 2719, e-mail:

aparissenti@hsnri.ca

SUPPLEMENTARY FIGURES

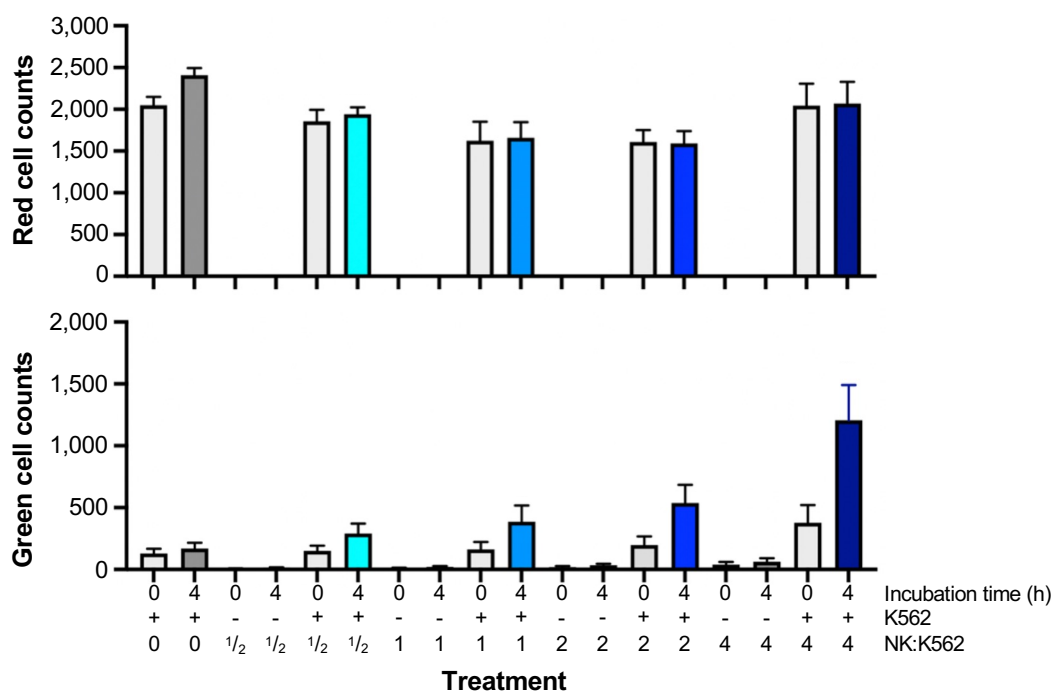


Supplementary Figure S1. Assessment of the purity of NK cell preparations. PBMCs were first isolated from human blood. NK cells were then enriched from the PMBC population. The purity of the NK cells was evaluated using flow cytometry. (a) Dot-plot of forward scatter (FSC) vs. side scatter (SSC). (b) Time plot. (c) Histogram of CD56 detection intensity. (d) Histogram of CD3 detection intensity. (e) Histogram of CD45 detection intensity. (f) Dot-plot of CD3 vs. CD56 fluorescence events gated for CD45+. Plots (showcasing PBMCs and enriched NK cells from a single individual) are representative of three separate human volunteers.

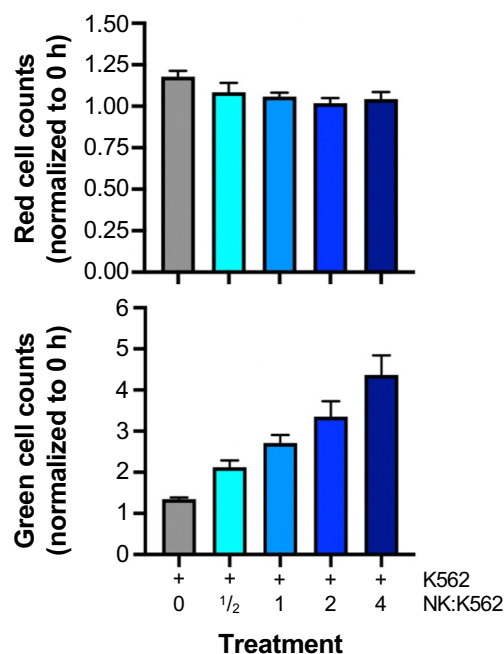


Supplementary Figure S2. Immunocytotoxicity of unstimulated NK cells on K562 cells as measured through loss of membrane integrity. K562 cells were co-cultured for 4 h with unstimulated NK cells at an NK:K562 ratio of 0.5:1, 1:1, 2:1 and 4:1. Live K562 cells were labelled with a cell-permeable red-fluorescing dye (red cells), whereas cells with a compromised plasma membrane were labelled with a cell-impermeable green-fluorescing dye (green cells). Labelled cells were visualized at 10 × magnification using a live-cell imaging and analysis system. Micrographs are representative of four independent biological experiments.

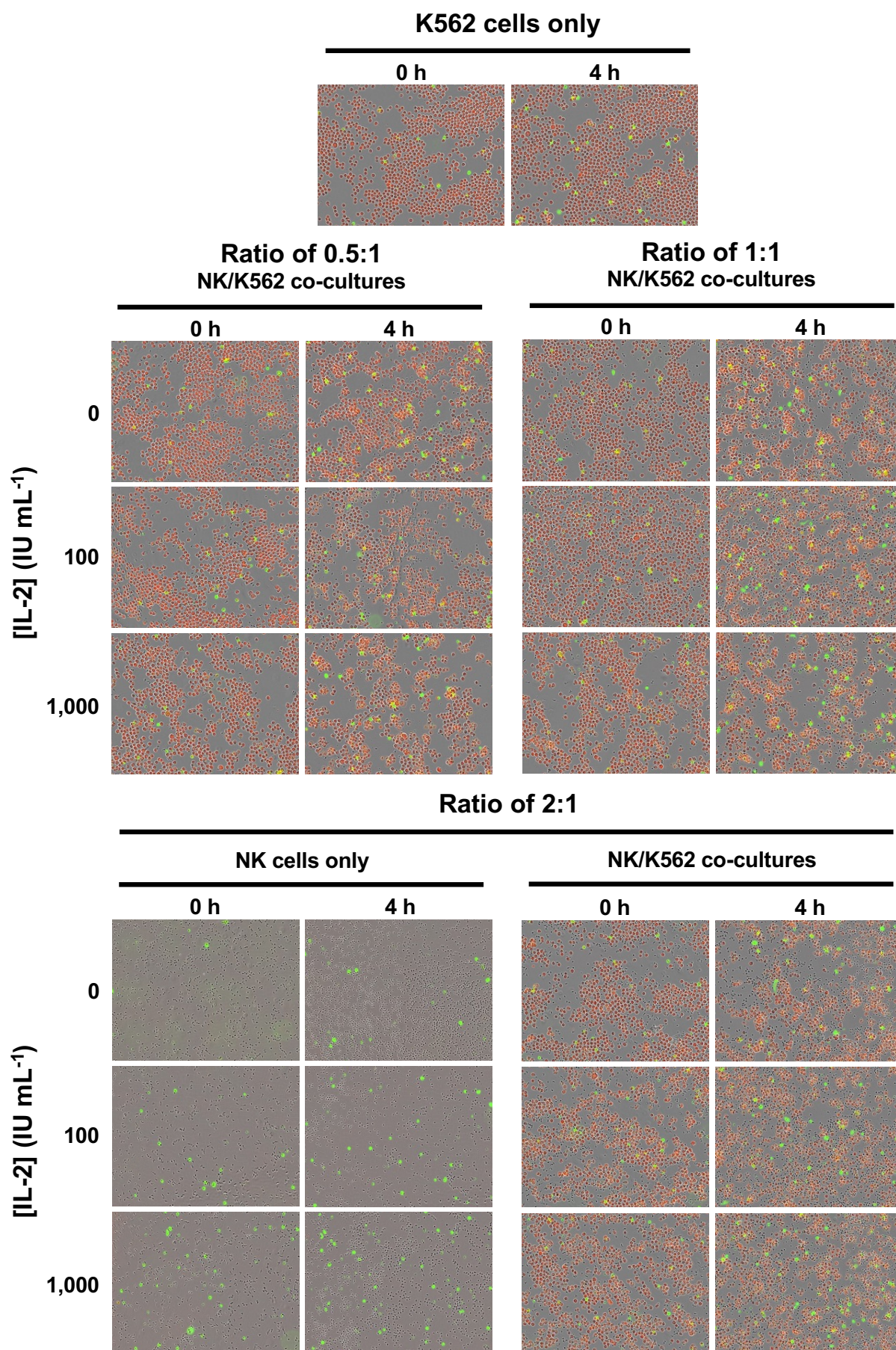
a)



b)

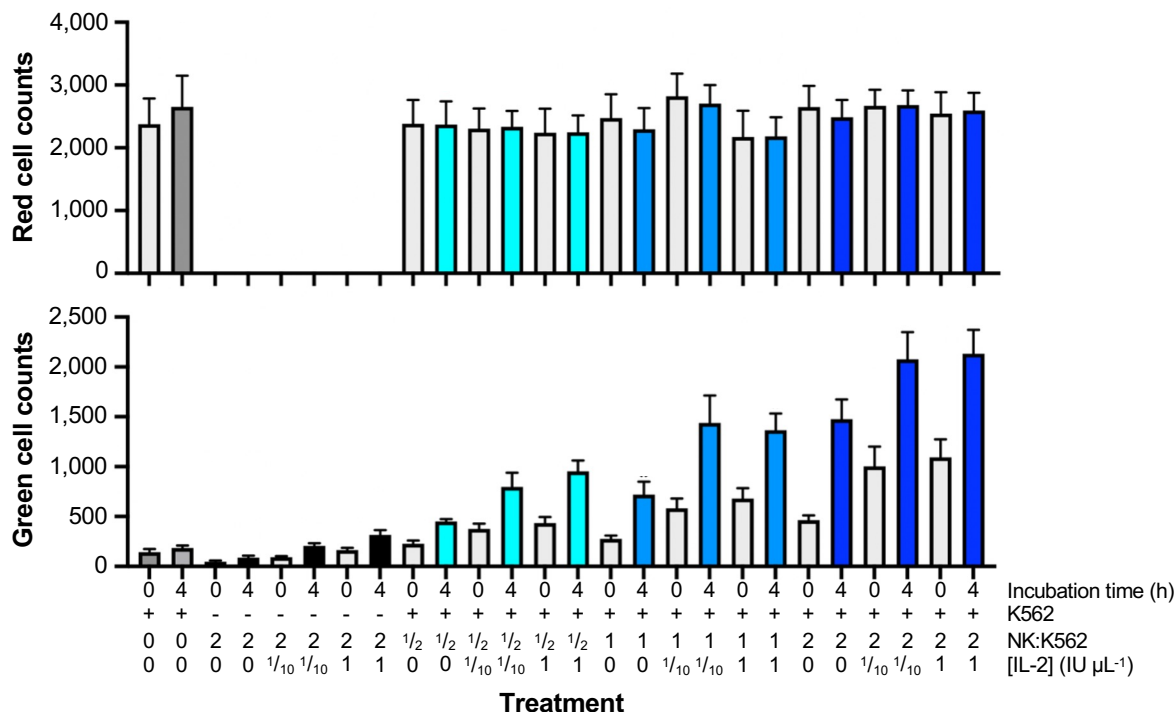


Supplementary Figure S3. Counts of K562 cells with intact and compromised plasma membranes following co-culture with unstimulated NK cells. K562 cells were co-cultured for 4 h with unstimulated NK cells at an NK:K562 ratio of 0.5:1, 1:1, 2:1 and 4:1. Live K562 cells were labelled with a cell-permeable red-fluorescing dye (red cells), whereas cells with a compromised plasma membrane were labelled with a cell-impermeable green-fluorescing dye (green cells). Labelled K562 cells were then counted using a live-cell imaging and analysis system. Raw counts after 0 and 4 h of incubation (**a**), and counts after 4 h of incubation normalized to counts after 0 h of incubation (**b**) are shown. Data are presented as means $\pm$ standard errors ($n = 4$).

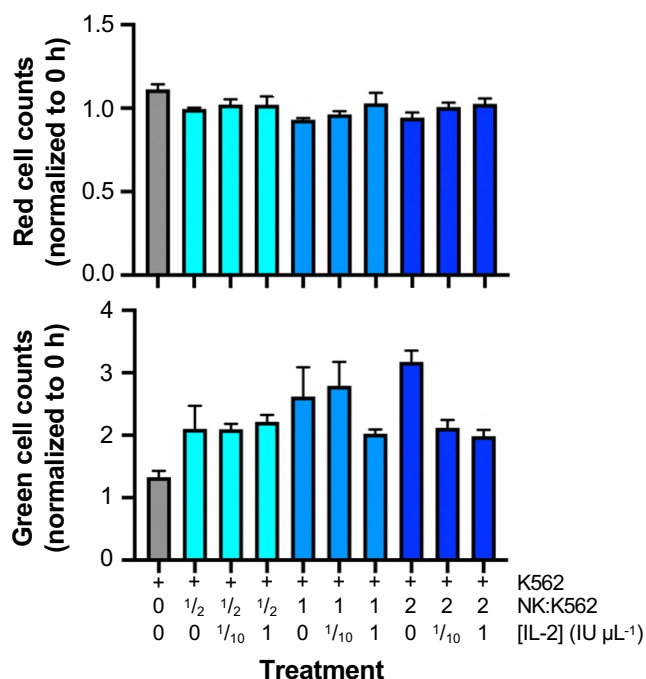


Supplementary Figure S4. Immunocytotoxicity of IL-2-activated NK cells on K562 cells as measured through loss of membrane integrity. K562 cells were co-cultured for 4 h with IL-2-activated NK cells at an NK:K562 ratio of 0.5:1, 1:1, 2:1 and 4:1. Live K562 cells were labelled with a cell-permeable red-fluorescing dye (red cells), whereas cells with a compromised plasma membrane were labelled with a cell-impermeable green-fluorescing dye (green cells). Labelled cells were visualized at 10 × magnification using a live-cell imaging and analysis system. Micrographs are representative of three independent biological experiments.

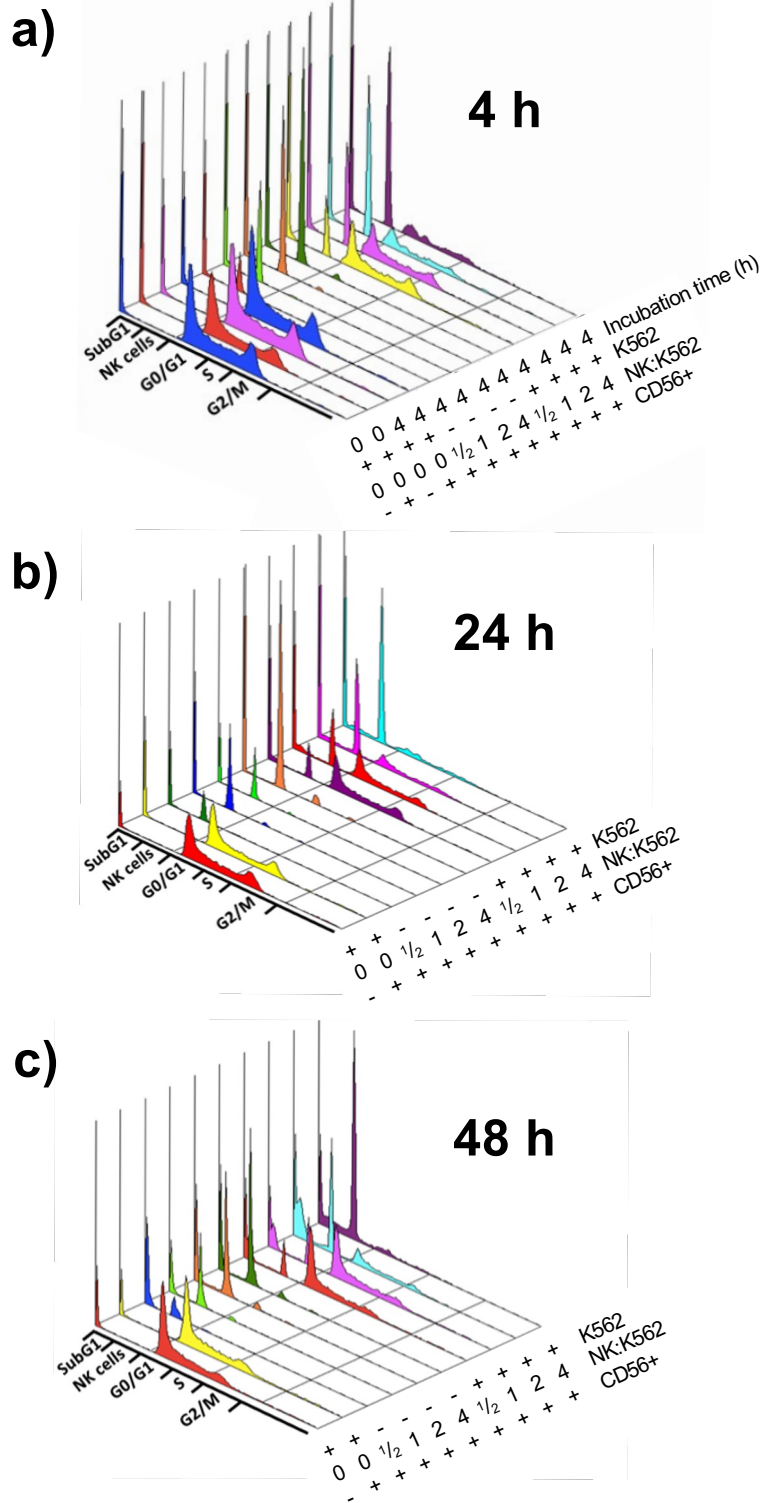
a)



b)



Supplementary Figure S5. Counts of K562 cells with intact and compromised plasma membranes following co-culture with IL-2-activated NK cells. K562 cells were co-cultured for 4 h with NK cells at an NK:K562 ratio of 0.5:1, 1:1, 2:1 and 4:1. When appropriate, NK cells were pre-activated with IL-2. Live K562 cells were labelled with a cell-permeable red-fluorescing dye (red cells), whereas cells with a compromised plasma membrane were labelled with a cell-impermeable green-fluorescing dye (green cells). Labelled K562 cells were then counted using a live-cell imaging and analysis system. Raw counts after 0 and 4 h of incubation (a), and counts after 4 h of incubation normalized to counts after 0 h of incubation (b) are shown. Data are presented as means $\pm$ standard errors ($n = 3$).

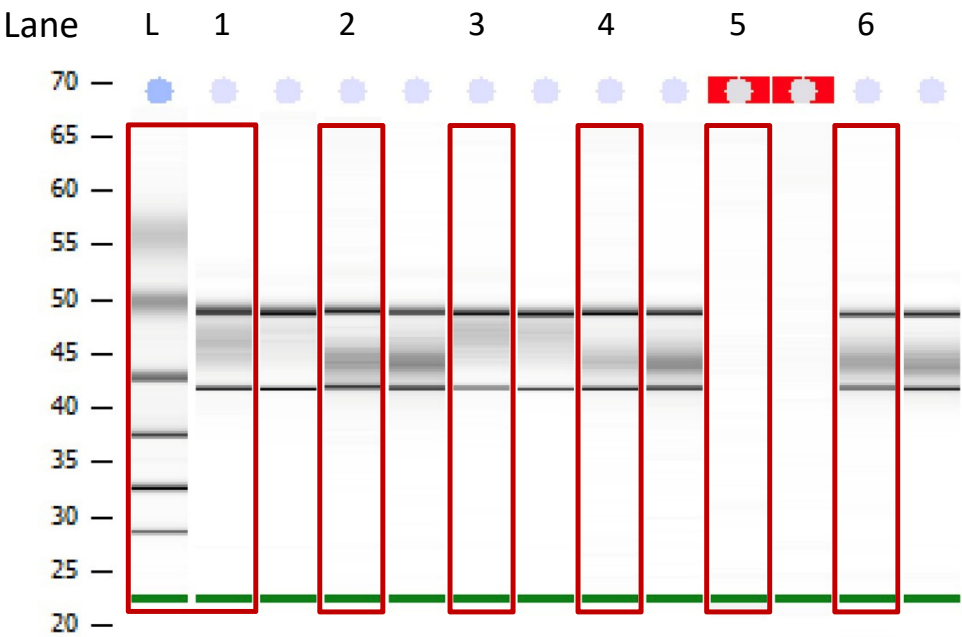


Supplementary Figure S6. DNA content analysis of K562 cells co-cultured with NK cells. K562 cells were incubated for 4 h (**a**), 24 h (**b**) or 48 h (**c**) with or without NK cells at an NK:K562 ratio of 0.5:1, 1:1, 2:1 and 4:1. In parallel, NK cells were cultured in the absence of K562 cells under identical conditions. When appropriate, NK cells were removed from cultures using CD56⁺ selection. The remaining K562 cells were then washed, fixed, stained with propidium iodide, and analyzed by flow cytometry. Peaks are labelled with the corresponding cell cycle stage. Each histogram is representative of three independent biological replicates.

**ORIGINAL AGILENT 2100 BIOANALYZER
ELECTROPHEROGRAM DATA FILES FOR MANUSCRIPT
FIGURES AND COMPOSITE IMAGES**

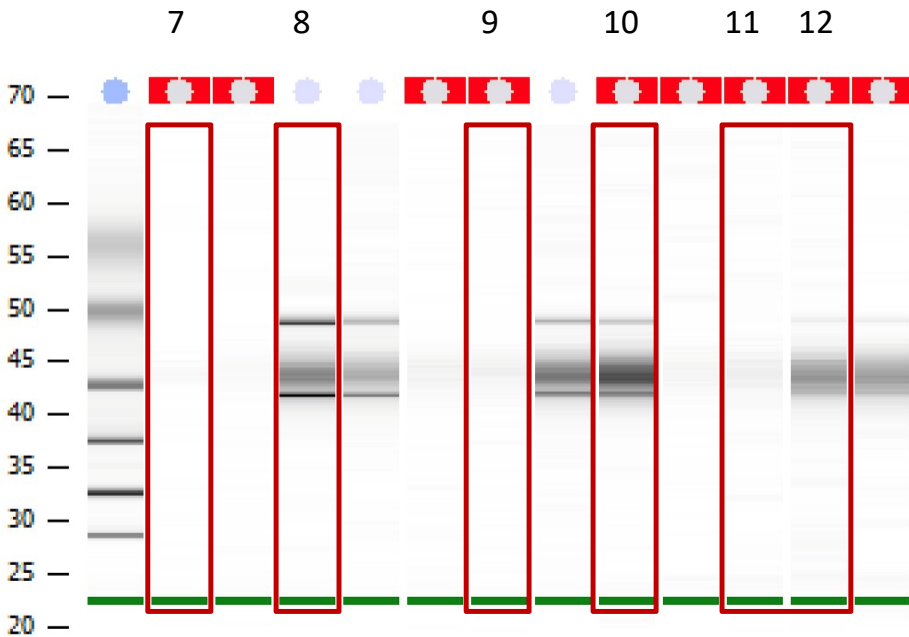
ORIGINAL DATA SUPPORTING COMPOSITE ELECTROPHEROGRAM IMAGES FOR FIGURE 1

Fig. 1. composite representative electropherogram



2100 Expert File Name: 2019-12-11_17-55-39

L = ladder

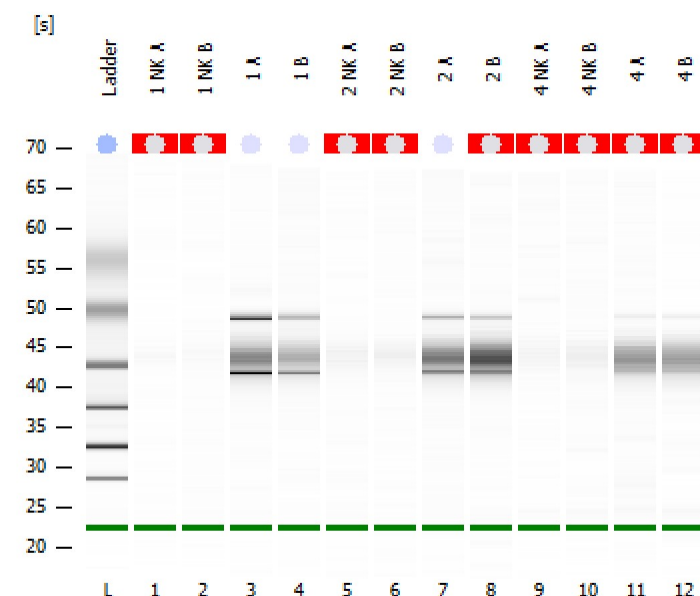


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Modified: 11/12/2019 4:53:16 PM

Electrophoresis File Run Summary



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Serial#: DEDAE00251

Firmware: C.01.069
Type: G2939B

Assay Information:

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Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

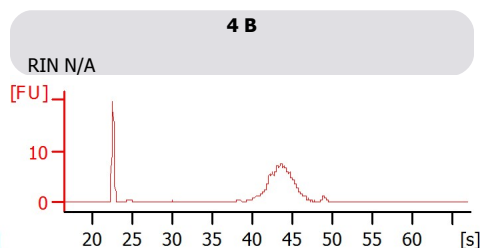
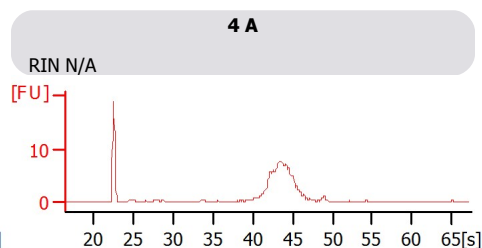
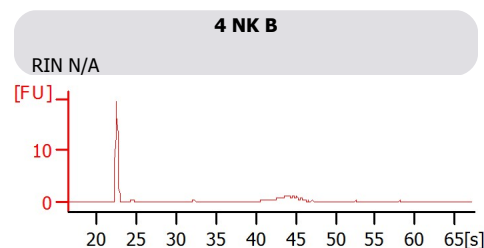
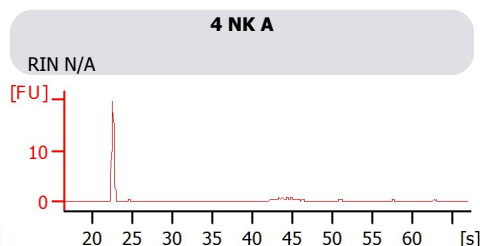
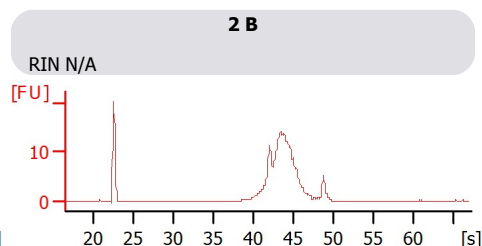
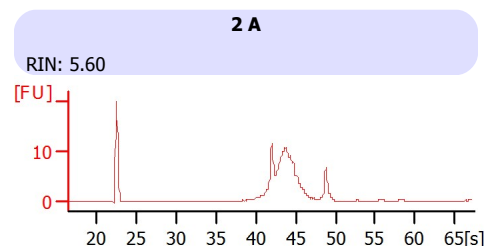
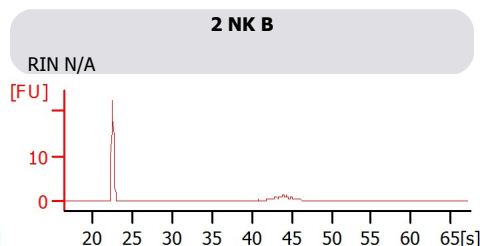
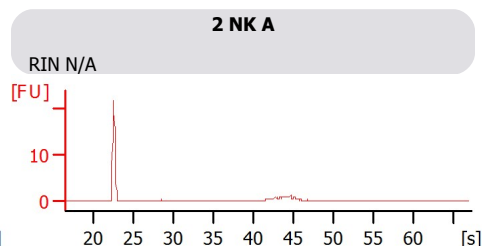
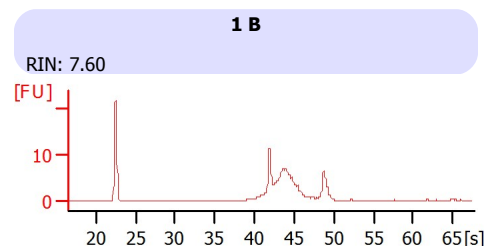
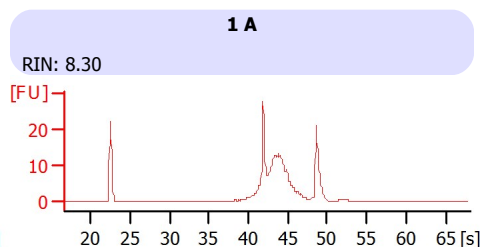
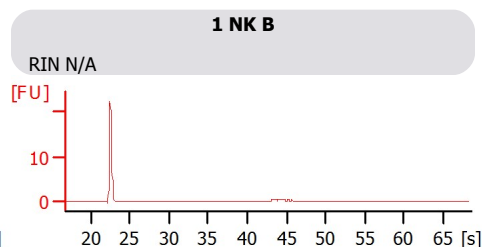
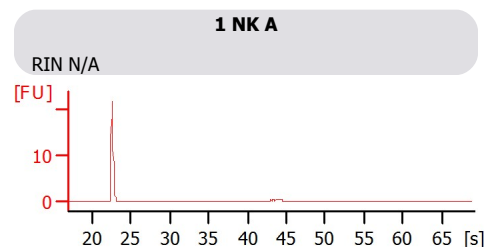
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Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments:



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_16-28-41.xad

Created: 11/12/2019 4:28:41 PM
Modified: 11/12/2019 4:53:16 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
1 NK A		✓	RIN N/A	
1 NK B		✓	RIN N/A	
1 A		✓	RIN: 8.30	
1 B		✓	RIN: 7.60	
2 NK A		✓	RIN N/A	
2 NK B		✓	RIN N/A	
2 A		✓	RIN: 5.60	
2 B		✓	RIN N/A	
4 NK A		✓	RIN N/A	
4 NK B		✓	RIN N/A	
4 A		✓	RIN N/A	
4 B		✓	RIN N/A	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_16-28-41.xad

Created: 11/12/2019 4:28:41 PM
Modified: 11/12/2019 4:53:16 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/ μ l] : 150

Lower Marker Concentration [ng/ μ l] : 0

Upper Marker Concentration [ng/ μ l] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

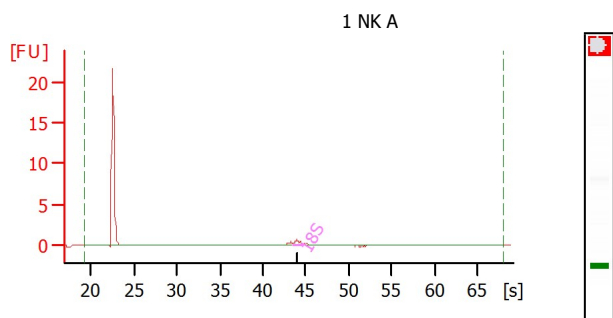
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_16-28-41.xad

Created: 11/12/2019 4:28:41 PM
Modified: 11/12/2019 4:53:16 PM

Electropherogram Summary

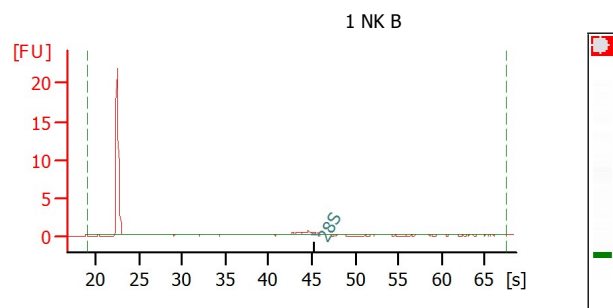


Overall Results for sample 1 : 1 NK A

RNA Area: 5.8
RNA Concentration: 3 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 1 : 1 NK A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.47	44.65	0.8	14.3

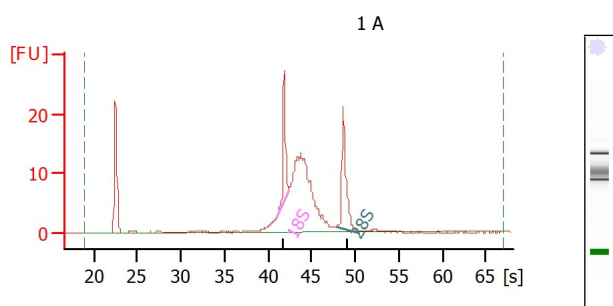


Overall Results for sample 2 : 1 NK B

RNA Area: 6.0
RNA Concentration: 3 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 2 : 1 NK B

Name	Start Time [s]	End Time [s]	Area	% of total Area
28S	45.05	45.44	0.1	2.4

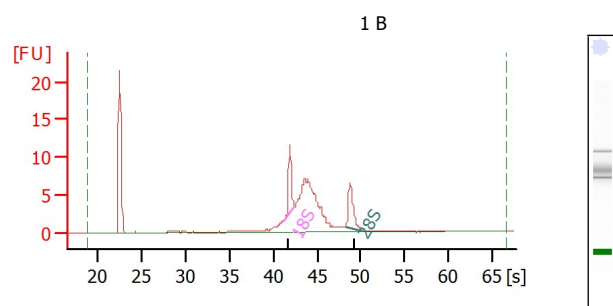


Overall Results for sample 3 : 1 A

RNA Area: 151.4
RNA Concentration: 81 ng/μl
rRNA Ratio [28s / 18s]: 1.3
RNA Integrity Number (RIN): 8.3 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.30

Fragment table for sample 3 : 1 A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.97	42.38	17.8	11.7
28S	47.92	50.39	23.3	15.4



Overall Results for sample 4 : 1 B

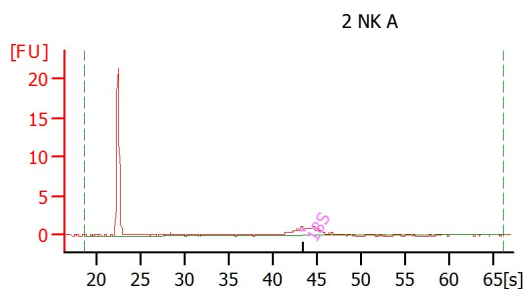
RNA Area: 71.2
RNA Concentration: 38 ng/μl
rRNA Ratio [28s / 18s]: 1.0
RNA Integrity Number (RIN): 7.6 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 7.60

Fragment table for sample 4 : 1 B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.28	42.44	7.6	10.7
28S	48.19	50.21	7.8	11.0

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_16-28-41.xad

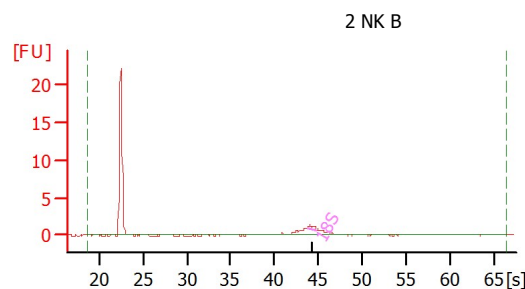
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Modified: 11/12/2019 4:53:16 PM

Electropherogram Summary Continued ...**Overall Results for sample 5 : 2 NK A**

RNA Area: 11.8
RNA Concentration: 6 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 5 : 2 NK A

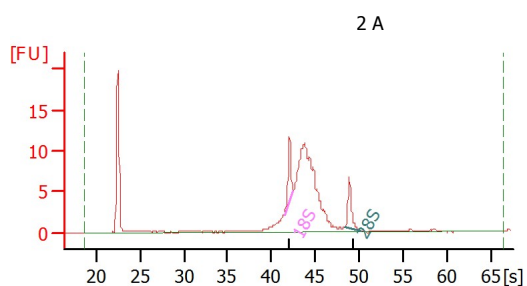
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.13	43.56	0.2	1.7

**Overall Results for sample 6 : 2 NK B**

RNA Area: 7.4
RNA Concentration: 4 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 6 : 2 NK B

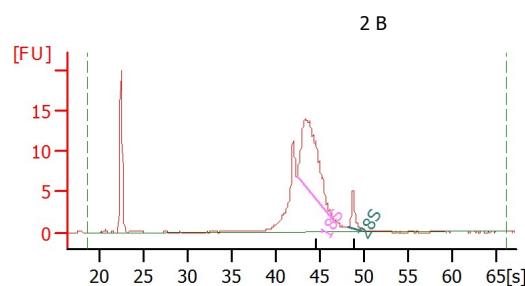
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.65	44.76	0.9	12.4

**Overall Results for sample 7 : 2 A**

RNA Area: 89.8
RNA Concentration: 48 ng/μl
rRNA Ratio [28s / 18s]: 0.9
RNA Integrity Number (RIN): 5.6 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 5.60

Fragment table for sample 7 : 2 A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.39	42.45	6.8	7.6
28S	48.32	50.14	6.0	6.7

**Overall Results for sample 8 : 2 B**

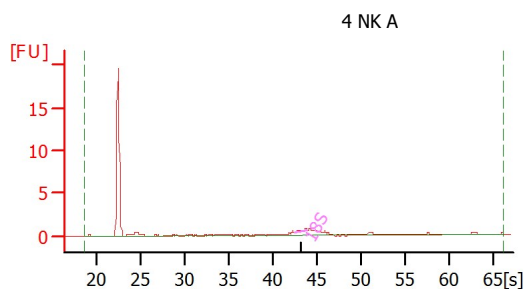
RNA Area: 120.5
RNA Concentration: 65 ng/μl
rRNA Ratio [28s / 18s]: 0.1
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 8 : 2 B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.46	46.58	42.8	35.5
28S	48.21	49.89	4.6	3.8

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_16-28-41.xad

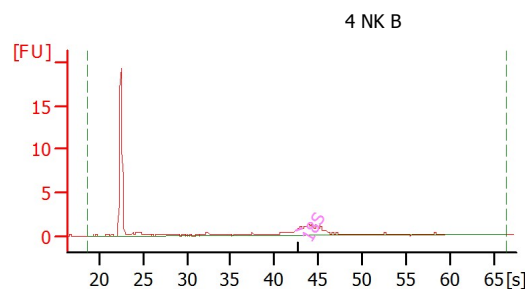
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Modified: 11/12/2019 4:53:16 PM

Electropherogram Summary Continued ...**Overall Results for sample 9 : 4 NK A**

RNA Area: 7.0
RNA Concentration: 4 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 9 : 4 NK A

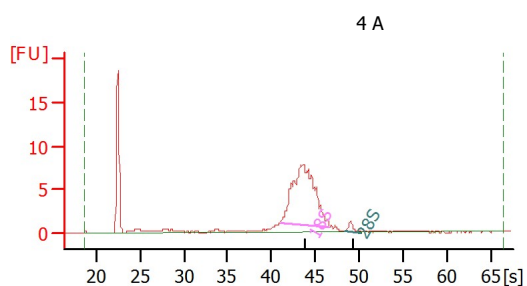
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.55	43.99	0.6	9.3

**Overall Results for sample 10 : 4 NK B**

RNA Area: 18.1
RNA Concentration: 10 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 10 : 4 NK B

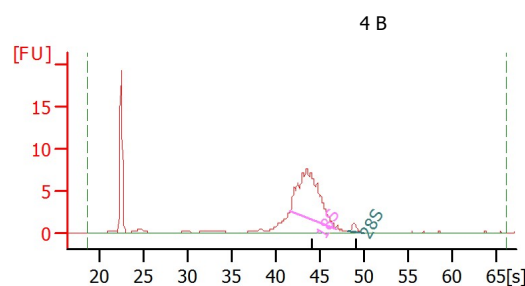
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.45	43.13	0.3	1.8

**Overall Results for sample 11 : 4 A**

RNA Area: 68.9
RNA Concentration: 37 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 11 : 4 A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.01	46.59	46.7	67.8
28S	48.37	50.24	1.3	1.8

**Overall Results for sample 12 : 4 B**

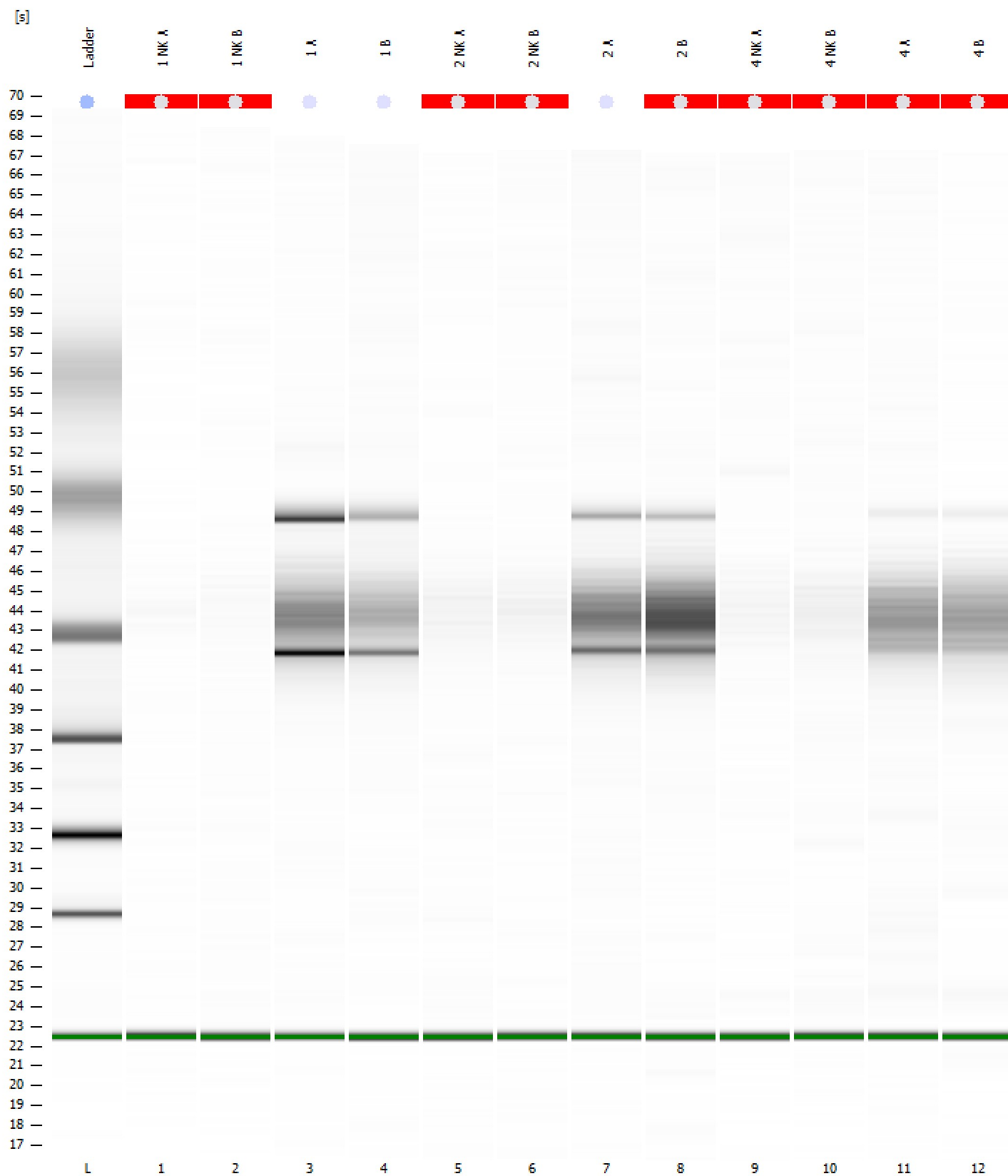
RNA Area: 72.0
RNA Concentration: 39 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 12 : 4 B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.69	46.78	36.7	51.0
28S	48.26	50.04	1.5	2.1

Assay Class: Eukaryote Total RNA Nano
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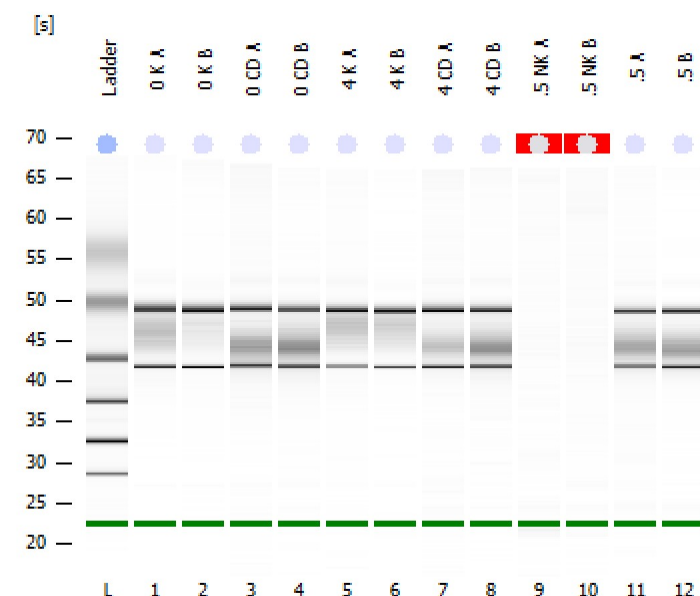
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Electrophoresis File Run Summary



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Instrument Name: DEDAE00251
Serial#: DEDAE00251

Firmware: C.01.069
Type: G2939B

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Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

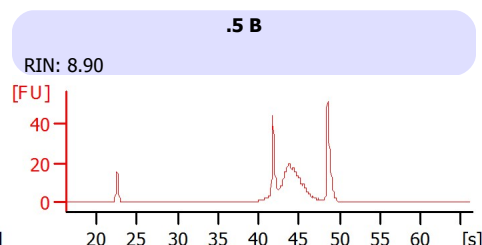
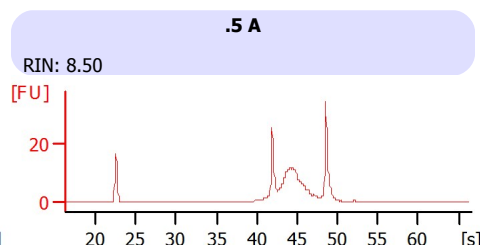
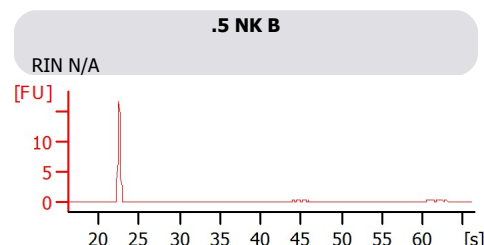
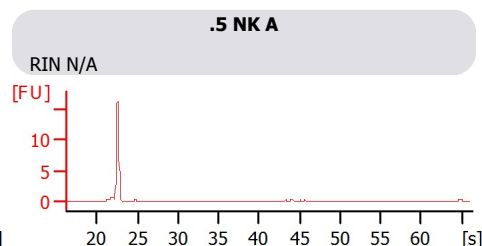
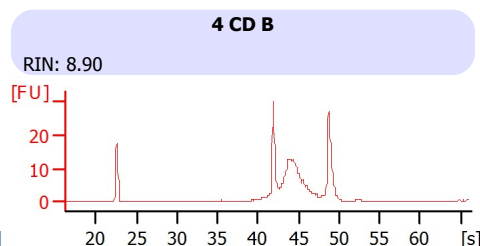
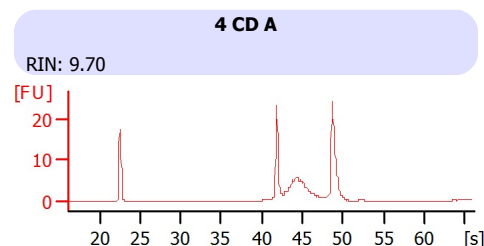
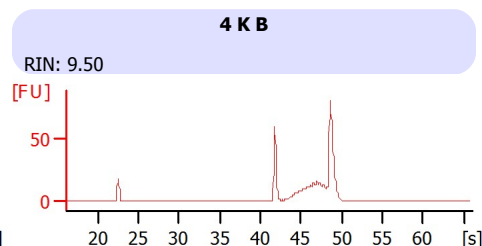
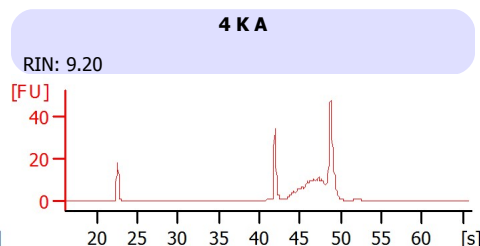
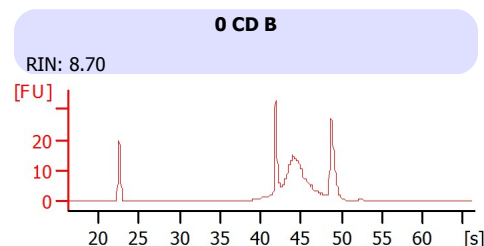
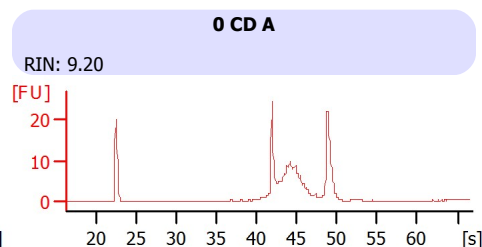
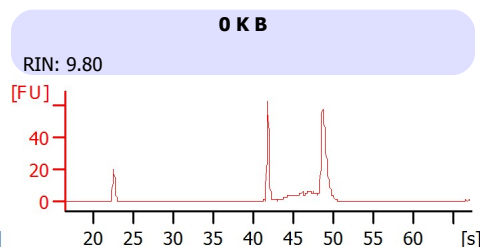
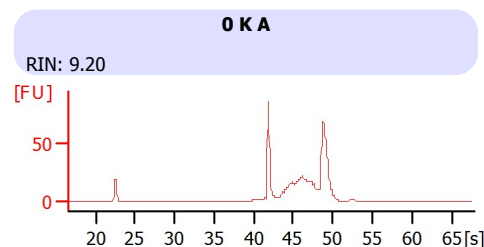
© Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments:



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

Created: 11/12/2019 5:55:39 PM
Modified: 11/12/2019 6:21:11 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
0 K A		✓	RIN: 9.20	
0 K B		✓	RIN: 9.80	
0 CD A		✓	RIN: 9.20	
0 CD B		✓	RIN: 8.70	
4 K A		✓	RIN: 9.20	
4 K B		✓	RIN: 9.50	
4 CD A		✓	RIN: 9.70	
4 CD B		✓	RIN: 8.90	
.5 NK A		✓	RIN N/A	
.5 NK B		✓	RIN N/A	
.5 A		✓	RIN: 8.50	
.5 B		✓	RIN: 8.90	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

Created: 11/12/2019 5:55:39 PM
Modified: 11/12/2019 6:21:11 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/μl] : 150

Lower Marker Concentration [ng/μl] : 0

Upper Marker Concentration [ng/μl] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

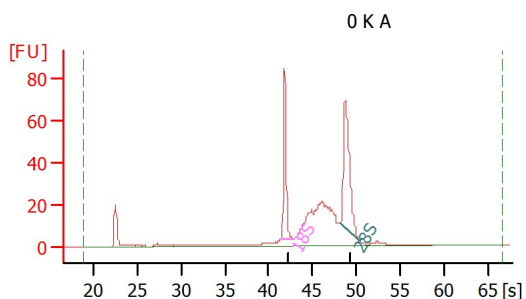
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

Created: 11/12/2019 5:55:39 PM
Modified: 11/12/2019 6:21:11 PM

Electropherogram Summary

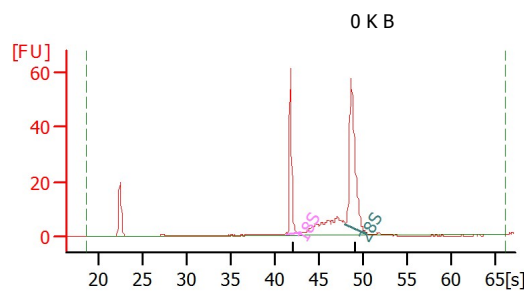


Overall Results for sample 1 : 0 K A

RNA Area: 382.2
RNA Concentration: 198 ng/μl
rRNA Ratio [28s / 18s]: 1.5
RNA Integrity Number (RIN): 9.2 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.20

Fragment table for sample 1 : 0 K A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.52	42.87	63.0	16.5
28S	48.24	50.56	96.3	25.2

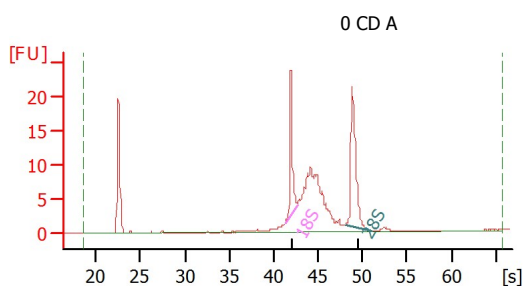


Overall Results for sample 2 : 0 K B

RNA Area: 186.0
RNA Concentration: 97 ng/μl
rRNA Ratio [28s / 18s]: 1.8
RNA Integrity Number (RIN): 9.8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.80

Fragment table for sample 2 : 0 K B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.50	42.99	43.9	23.6
28S	48.07	50.52	79.7	42.9

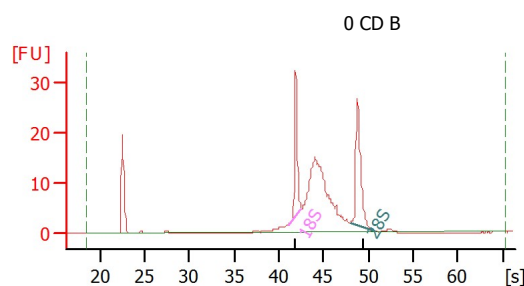


Overall Results for sample 3 : 0 CD A

RNA Area: 117.4
RNA Concentration: 61 ng/μl
rRNA Ratio [28s / 18s]: 1.6
RNA Integrity Number (RIN): 9.2 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.20

Fragment table for sample 3 : 0 CD A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.28	42.71	17.1	14.6
28S	48.05	50.96	27.4	23.4



Overall Results for sample 4 : 0 CD B

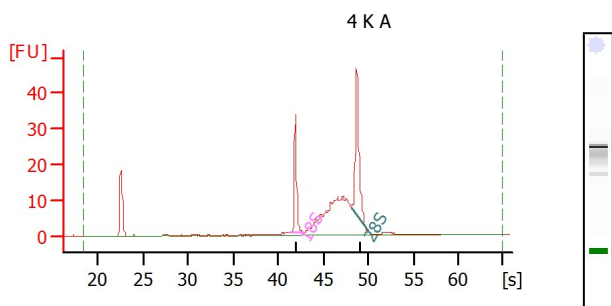
RNA Area: 171.0
RNA Concentration: 89 ng/μl
rRNA Ratio [28s / 18s]: 1.4
RNA Integrity Number (RIN): 8.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.70

Fragment table for sample 4 : 0 CD B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.02	42.54	22.5	13.2
28S	48.03	50.64	31.0	18.1

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

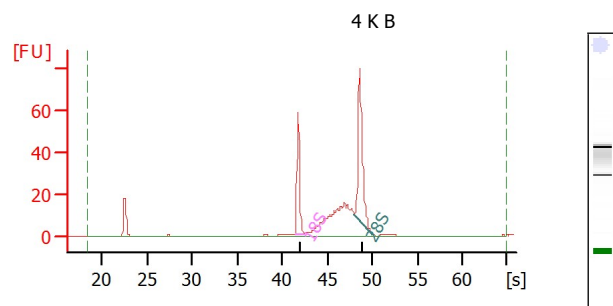
Created: 11/12/2019 5:55:39 PM
Modified: 11/12/2019 6:21:11 PM

Electropherogram Summary Continued ...**Overall Results for sample 5 : 4 K A**

RNA Area: 165.6
RNA Concentration: 86 ng/μl
rRNA Ratio [28s / 18s]: 1.7
RNA Integrity Number (RIN): 9.2 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.20

Fragment table for sample 5 : 4 K A

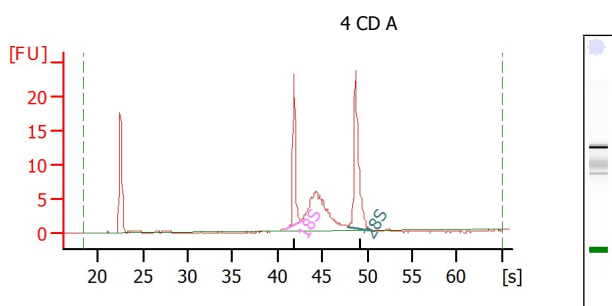
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.23	42.83	24.2	14.6
28S	48.15	49.90	41.5	25.0

**Overall Results for sample 6 : 4 K B**

RNA Area: 260.2
RNA Concentration: 135 ng/μl
rRNA Ratio [28s / 18s]: 1.9
RNA Integrity Number (RIN): 9.5 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.50

Fragment table for sample 6 : 4 K B

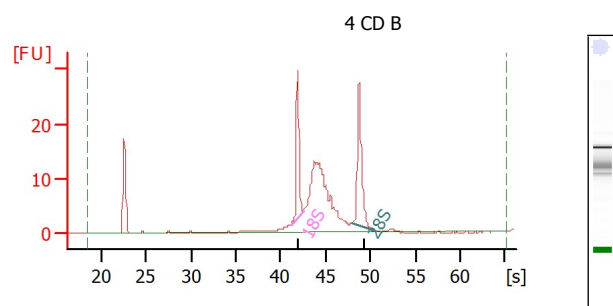
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.47	42.51	41.9	16.1
28S	48.01	49.94	80.0	30.8

**Overall Results for sample 7 : 4 CD A**

RNA Area: 85.2
RNA Concentration: 44 ng/μl
rRNA Ratio [28s / 18s]: 1.7
RNA Integrity Number (RIN): 9.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.70

Fragment table for sample 7 : 4 CD A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.99	42.69	16.1	18.9
28S	47.78	50.75	27.4	32.2

**Overall Results for sample 8 : 4 CD B**

RNA Area: 144.1
RNA Concentration: 75 ng/μl
rRNA Ratio [28s / 18s]: 1.4
RNA Integrity Number (RIN): 8.9 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.90

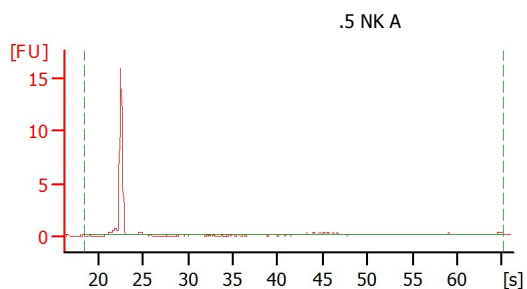
Fragment table for sample 8 : 4 CD B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.17	42.54	21.0	14.6
28S	47.84	50.58	29.2	20.3

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

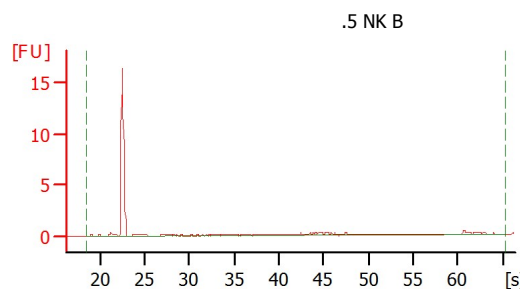
Created: 11/12/2019 5:55:39 PM
 Modified: 11/12/2019 6:21:11 PM

Electropherogram Summary Continued ...



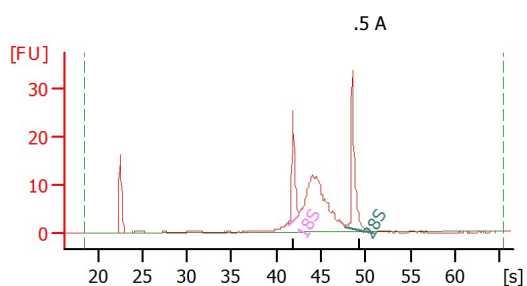
Overall Results for sample 9 : .5 NK A

RNA Area: 3.8
 RNA Concentration: 2 ng/μl
 rRNA Ratio [28s / 18s]: 0.0
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A



Overall Results for sample 10 : .5 NK B

RNA Area: 5.9
 RNA Concentration: 3 ng/μl
 rRNA Ratio [28s / 18s]: 0.0
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

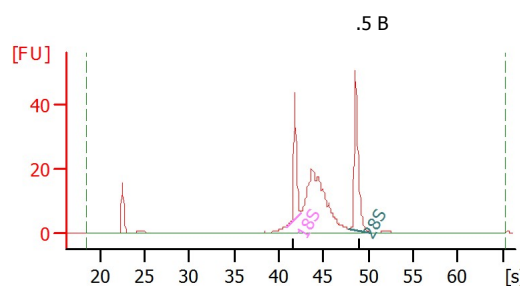


Overall Results for sample 11 : .5 A

RNA Area: 148.3
 RNA Concentration: 77 ng/μl
 rRNA Ratio [28s / 18s]: 1.5
 RNA Integrity Number (RIN): 8.5 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.50

Fragment table for sample 11 : .5 A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.34	42.58	19.2	13.0
28S	47.75	50.74	28.8	19.4



Overall Results for sample 12 : .5 B

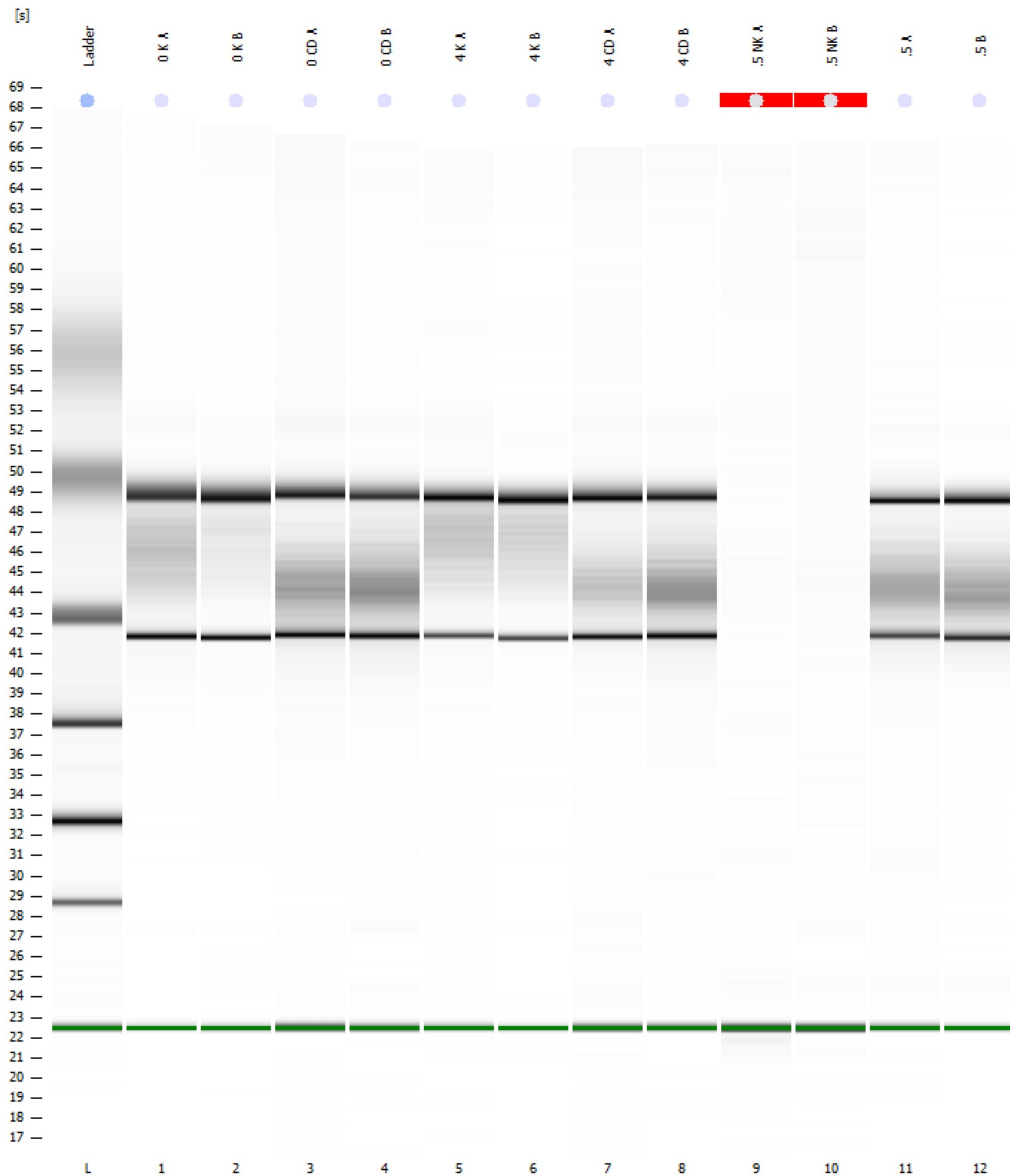
RNA Area: 225.4
 RNA Concentration: 117 ng/μl
 rRNA Ratio [28s / 18s]: 1.5
 RNA Integrity Number (RIN): 8.9 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.90

Fragment table for sample 12 : .5 B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.88	42.44	32.6	14.5
28S	47.79	50.26	49.6	22.0

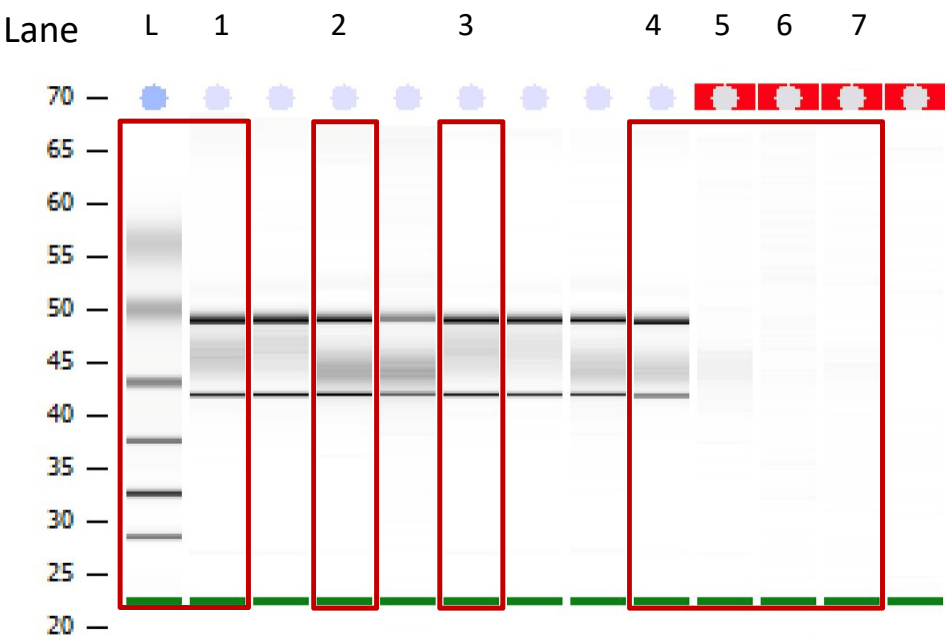
Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2019-12-11_17-55-39.xad

Created: 11/12/2019 5:55:39 PM
Modified: 11/12/2019 6:21:11 PM

Gel Image

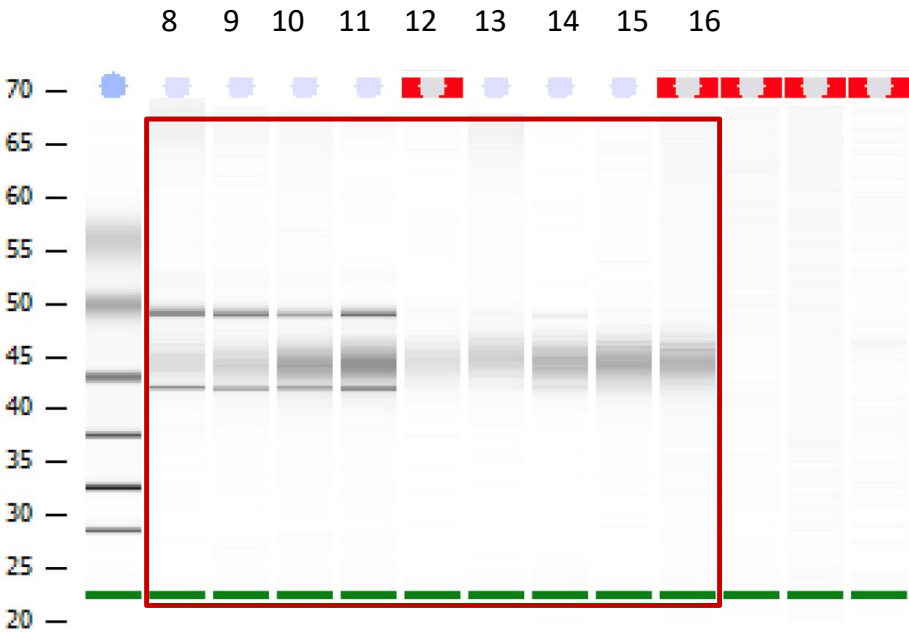
ORIGINAL DATA SUPPORTING COMPOSITE ELECTROPHEROGRAM IMAGES FOR FIGURE 2

Fig. 2. composite representative electropherogram



2100 Expert File Name: 2020-02-22_17-59-45

L = ladder

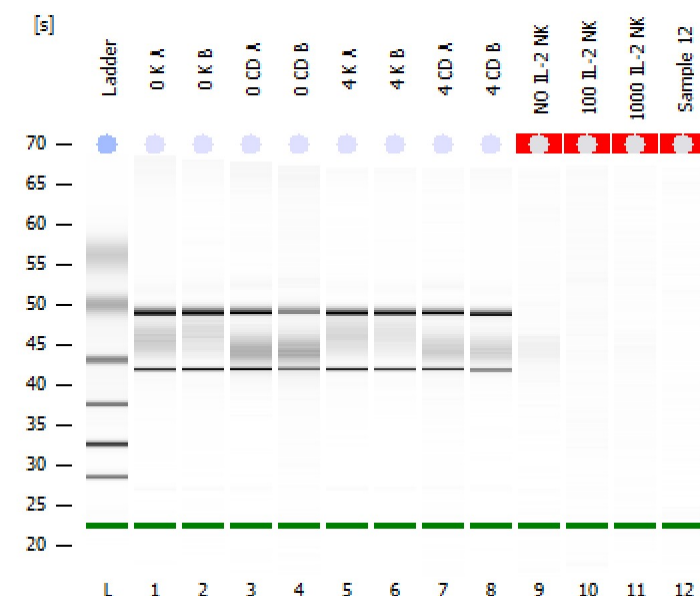


2100 Expert File Name: 2020-02-22_18-36-48

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DEDAE00251
Serial#: DEDAE00251

Firmware: C.01.069
Type: G2939B

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

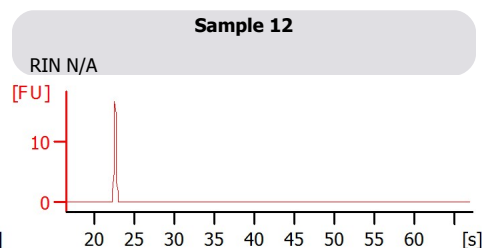
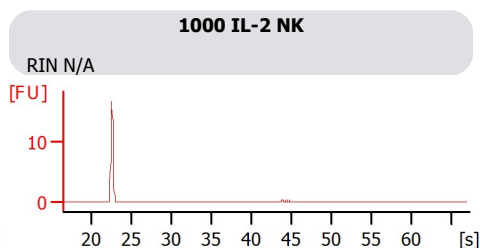
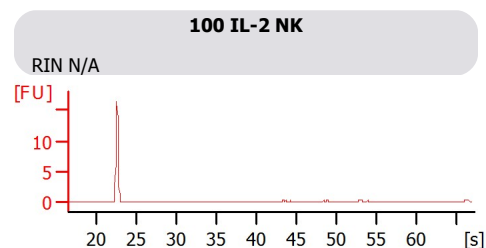
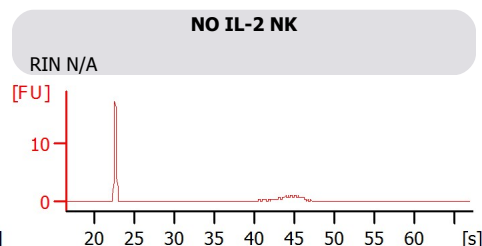
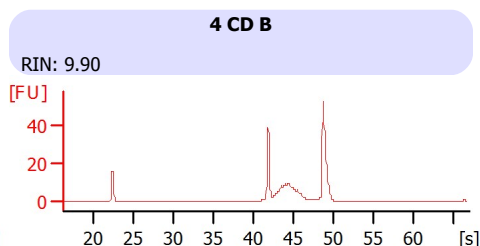
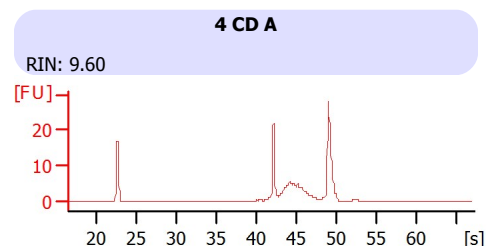
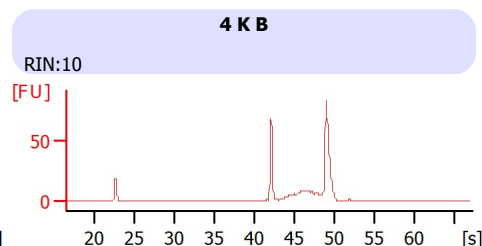
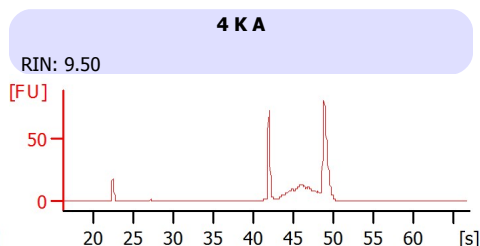
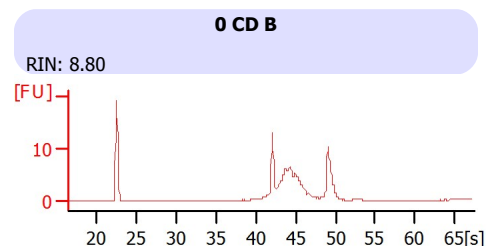
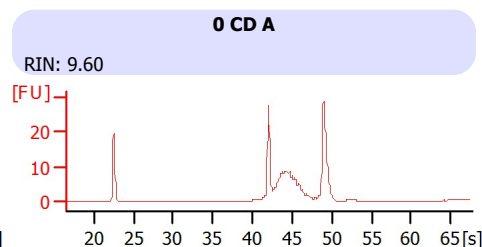
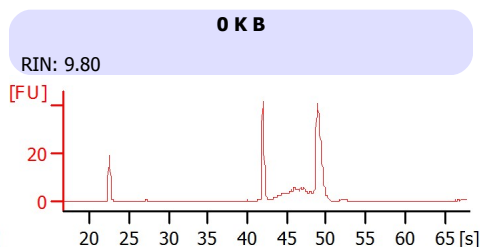
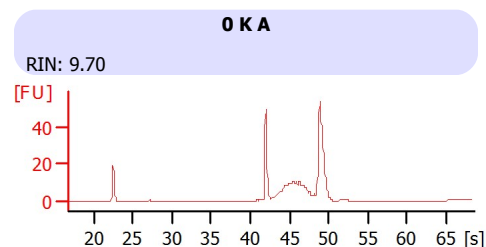
© Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments:



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
0 K A		✓	RIN: 9.70	
0 K B		✓	RIN: 9.80	
0 CD A		✓	RIN: 9.60	
0 CD B		✓	RIN: 8.80	
4 K A		✓	RIN: 9.50	
4 K B		✓	RIN:10	
4 CD A		✓	RIN: 9.60	
4 CD B		✓	RIN: 9.90	
NO IL-2 NK		✓	RIN N/A	
100 IL-2 NK		✓	RIN N/A	
1000 IL-2 NK		✓	RIN N/A	
Sample 12		✓	RIN N/A	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13
Minimum Visible Range [s] : 17
Maximum Visible Range [s] : 70
Start Analysis Time Range [s] : 19
End Analysis Time Range [s] : 69
Ladder Concentration [ng/ μ l] : 150
Lower Marker Concentration [ng/ μ l] : 0
Upper Marker Concentration [ng/ μ l] : 0
Used Lower Marker for Quantitation
Standard Curve Fit is Logarithmic
Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19
Integration End Time [s] : 69
Slope Threshold : 0.6
Height Threshold [FU] : 0.5
Area Threshold : 0.2
Width Threshold [s] : 0.5
Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5
Polynomial Order : 4

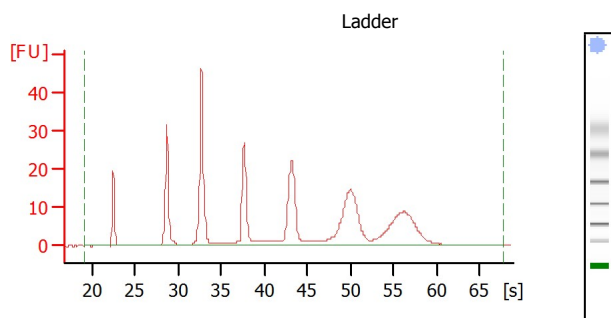
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

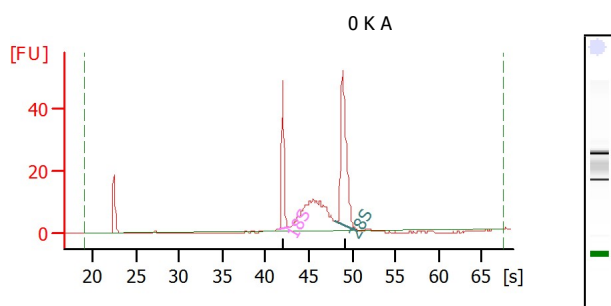
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area: 366.6
RNA Concentration: 150 ng/μl
Result Flagging Color:
Result Flagging Label: All Other Samples



Overall Results for sample 1 : 0 K A

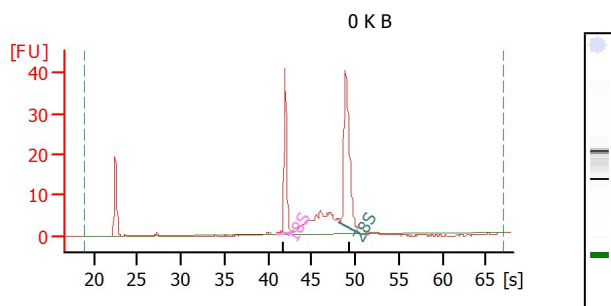
RNA Area: 198.7
RNA Concentration: 81 ng/μl
rRNA Ratio [28s / 18s]: 2.0
RNA Integrity Number (RIN): 9.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.70

Fragment table for sample 1 : 0 K A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.63	42.60	37.4	18.8
28S	47.89	50.58	74.7	37.6

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

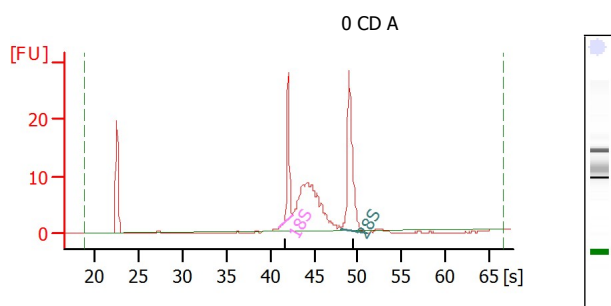
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 2 : 0 K B**

RNA Area: 143.1
RNA Concentration: 59 ng/μl
rRNA Ratio [28s / 18s]: 1.8
RNA Integrity Number (RIN): 9.8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.80

Fragment table for sample 2 : 0 K B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.02	42.72	30.9	21.6
28S	48.26	50.59	55.4	38.7

**Overall Results for sample 3 : 0 CD A**

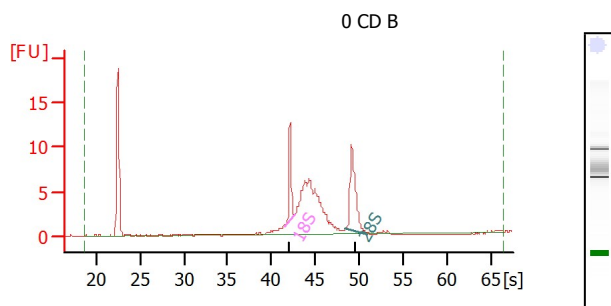
RNA Area: 119.0
RNA Concentration: 49 ng/μl
rRNA Ratio [28s / 18s]: 2.0
RNA Integrity Number (RIN): 9.6 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.60

Fragment table for sample 3 : 0 CD A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.09	42.54	18.7	15.7
28S	48.04	51.04	37.8	31.7

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

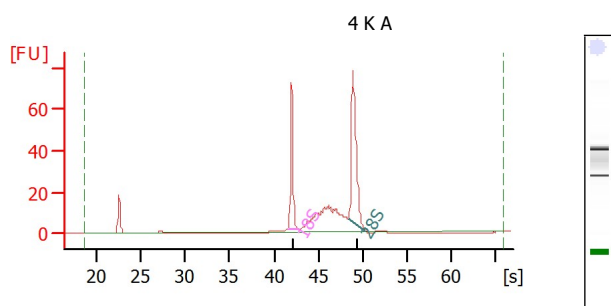
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 4 : 0 CD B**

RNA Area: 68.4
RNA Concentration: 28 ng/μl
rRNA Ratio [28s / 18s]: 1.7
RNA Integrity Number (RIN): 8.8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.80

Fragment table for sample 4 : 0 CD B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.54	42.60	8.1	11.9
28S	48.41	50.82	14.0	20.4

**Overall Results for sample 5 : 4 K A**

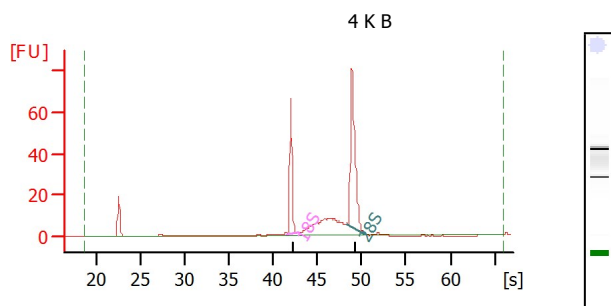
RNA Area: 269.9
RNA Concentration: 110 ng/μl
rRNA Ratio [28s / 18s]: 1.7
RNA Integrity Number (RIN): 9.5 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.50

Fragment table for sample 5 : 4 K A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.66	42.85	54.9	20.3
28S	48.34	50.30	92.6	34.3

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

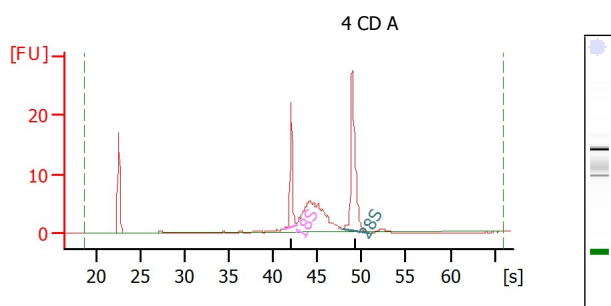
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 6 : 4 K B**

RNA Area: 225.0
RNA Concentration: 92 ng/μl
rRNA Ratio [28s / 18s]: 1.9
RNA Integrity Number (RIN): 10 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN:10

Fragment table for sample 6 : 4 K B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.65	42.99	49.4	21.9
28S	48.40	50.41	93.6	41.6

**Overall Results for sample 7 : 4 CD A**

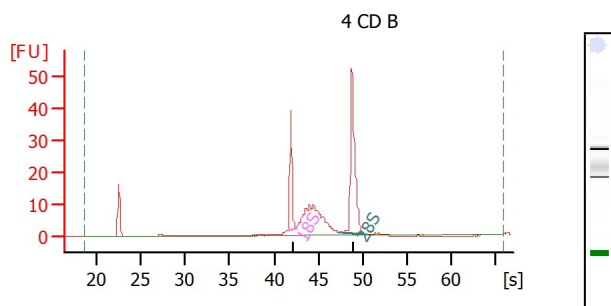
RNA Area: 92.3
RNA Concentration: 38 ng/μl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 9.6 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.60

Fragment table for sample 7 : 4 CD A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.22	42.70	15.1	16.3
28S	47.87	50.79	32.0	34.6

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

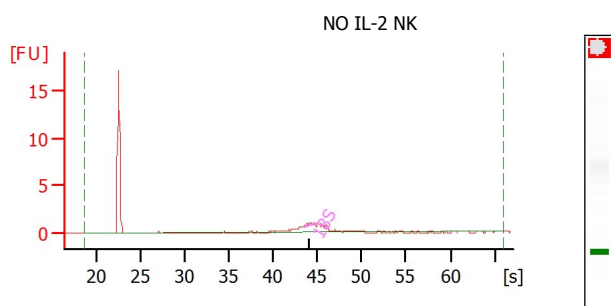
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 8 : 4 CD B**

RNA Area: 150.3
RNA Concentration: 61 ng/μl
rRNA Ratio [28s / 18s]: 2.3
RNA Integrity Number (RIN): 9.9 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.90

Fragment table for sample 8 : 4 CD B

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.61	42.47	26.3	17.5
28S	47.44	50.45	60.9	40.5

**Overall Results for sample 9 : NO IL-2 NK**

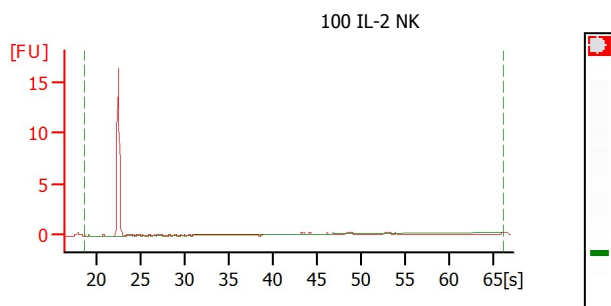
RNA Area: 7.6
RNA Concentration: 3 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 9 : NO IL-2 NK

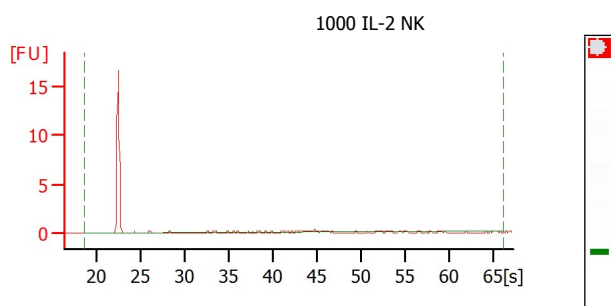
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.66	44.62	0.3	4.3

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 10 : 100 IL-2 NK**

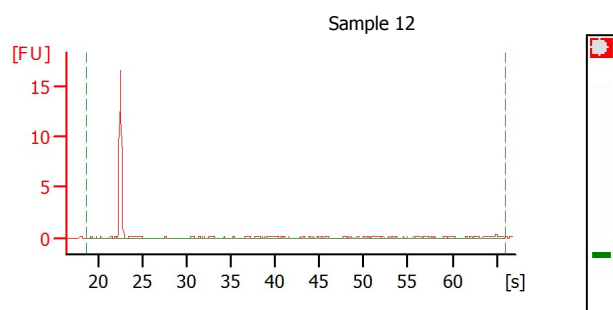
RNA Area: 1.1
RNA Concentration: 0 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

**Overall Results for sample 11 : 1000 IL-2 NK**

RNA Area: 7.6
RNA Concentration: 3 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

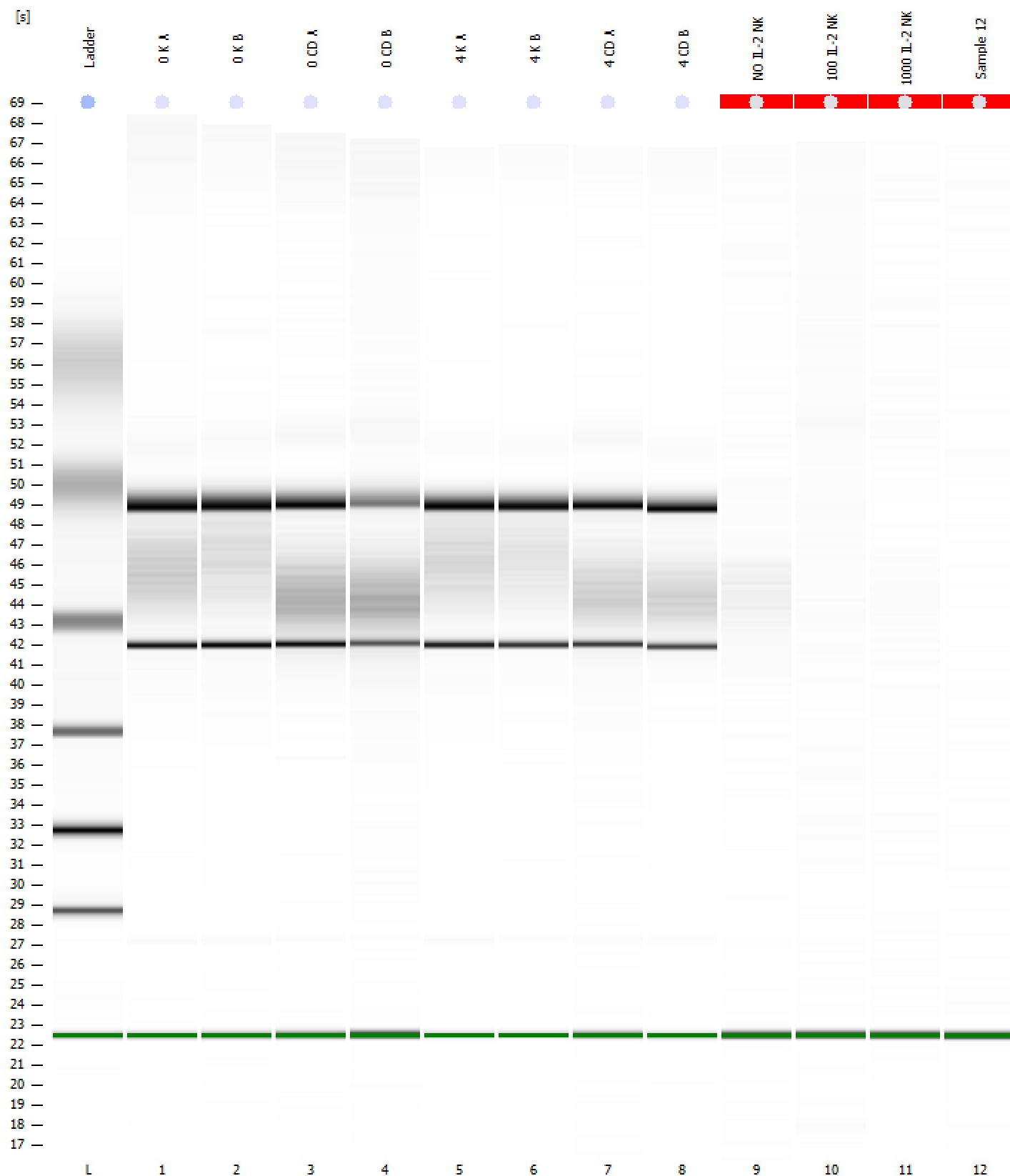
Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Electropherogram Summary Continued ...**Overall Results for sample 12 : Sample 12**

RNA Area: 3.1
RNA Concentration: 1 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

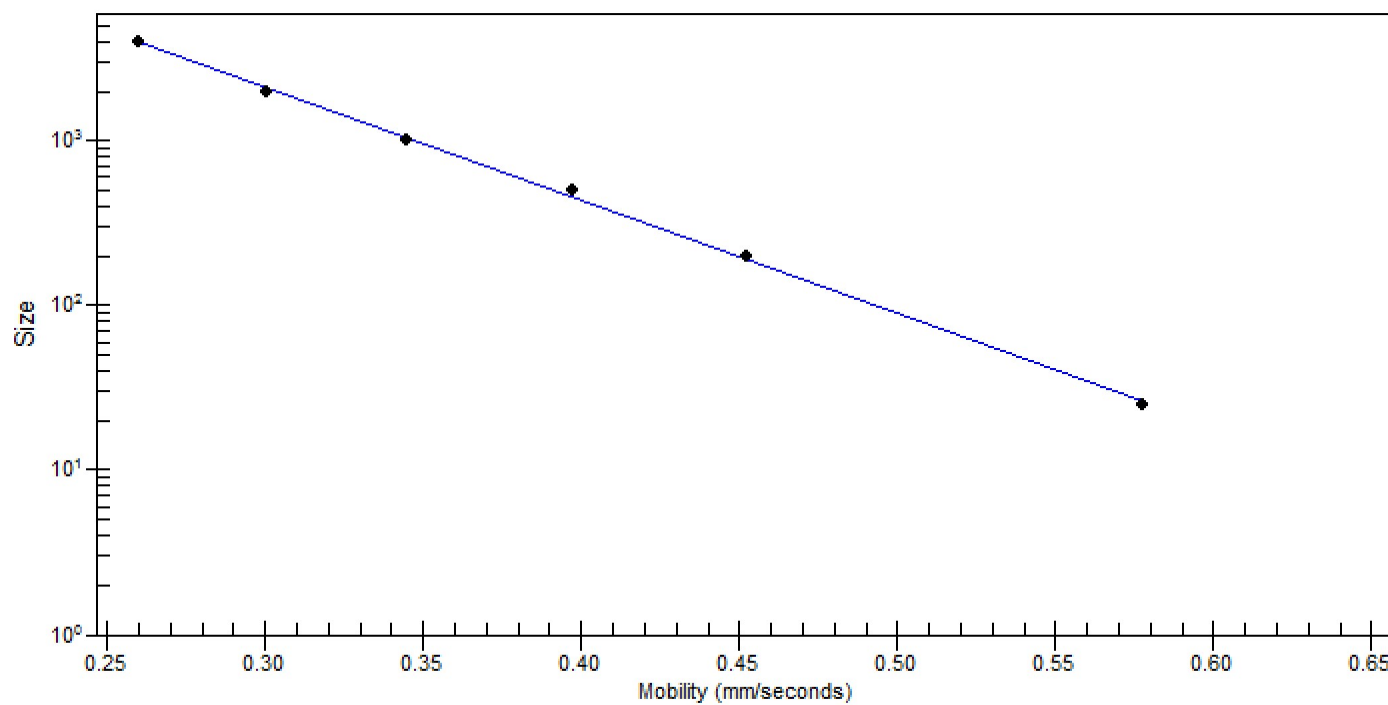
Gel Image

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
Modified: 22/02/2020 6:25:52 PM

Curves

Standard Curve



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad

Created: 22/02/2020 5:59:45 PM
 Modified: 22/02/2020 6:25:52 PM

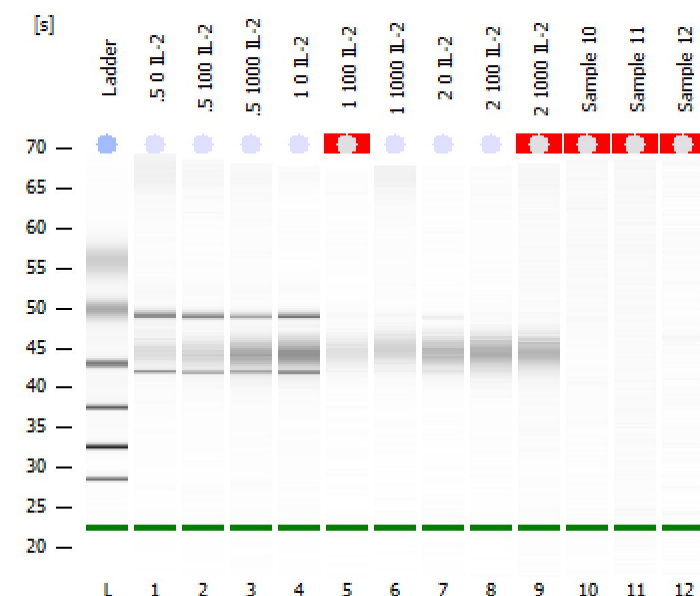
Run Logbook

Description	Number	Source	Category	Sub Category	Time	Time Zone	User	Host
Run ended on port 1 (Number of wells acquired: 13)		Instrument	Run		22/02/2020 6:22:47 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Run started on port 1 (File: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\Data\2020-02-22\2100 expert_Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_17-59-45.xad)		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Product Number : G2939B		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Name :		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Vendor : Agilent Technologies		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Serial# : DEDAE00251		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Firmware : C.01.069		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Cartridge : Electrode		Instrument	Run		22/02/2020 5:59:50 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electrophoresis File Run Summary



Instrument Information:

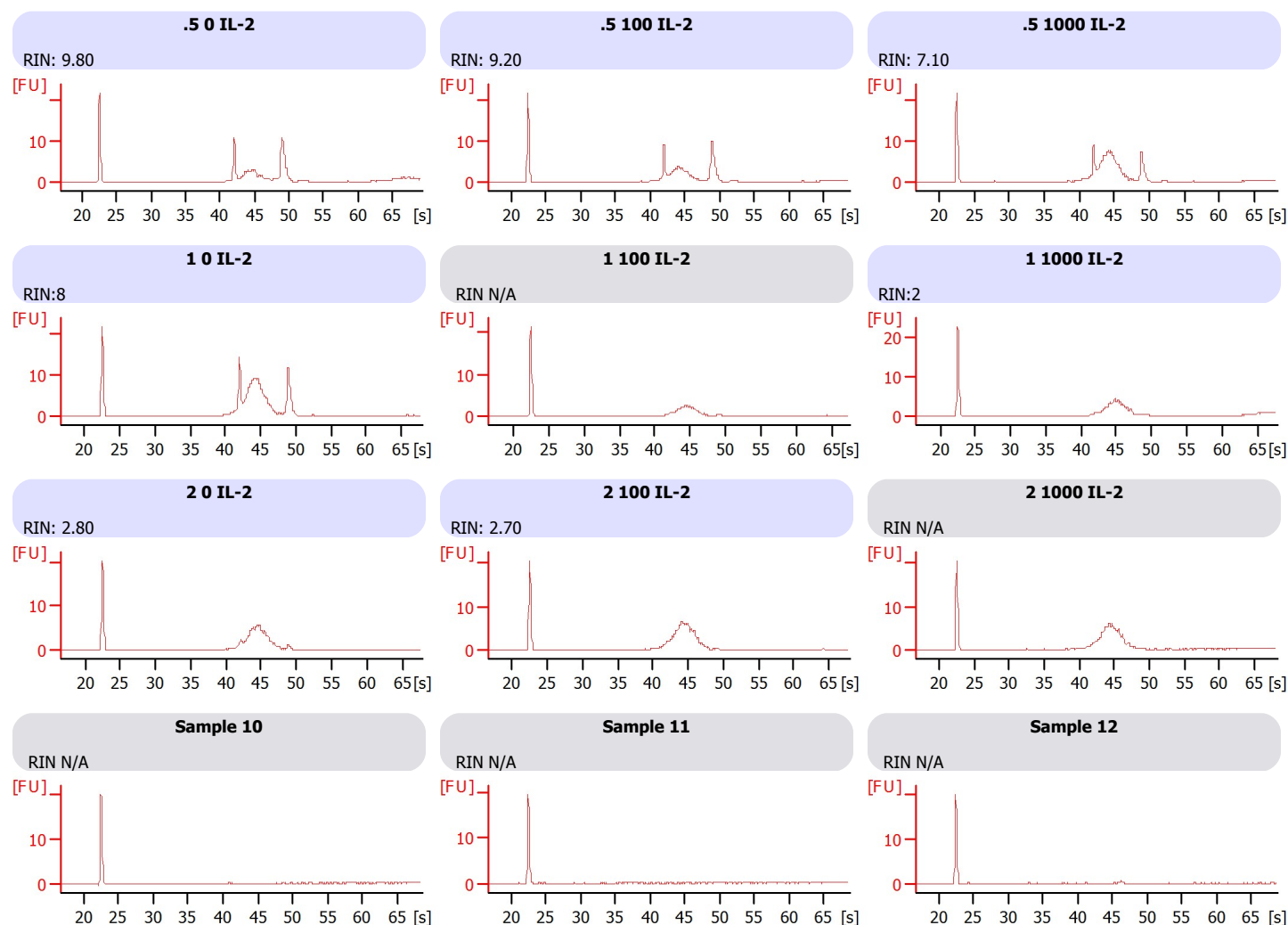
Instrument Name: DEDAE00251
Serial#: DEDAE00251
Firmware: C.01.069
Type: G2939B

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy
Assay Class: Eukaryote Total RNA Nano
Version: 2.6
Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)
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Chip Information:

Chip Lot #:
Reagent Kit Lot #:
Chip Comments:



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
.5 0 IL-2		✓	RIN: 9.80	
.5 100 IL-2		✓	RIN: 9.20	
.5 1000 IL-2		✓	RIN: 7.10	
1 0 IL-2		✓	RIN:8	
1 100 IL-2		✓	RIN N/A	
1 1000 IL-2		✓	RIN:2	
2 0 IL-2		✓	RIN: 2.80	
2 100 IL-2		✓	RIN: 2.70	
2 1000 IL-2		✓	RIN N/A	
Sample 10		✓	RIN N/A	
Sample 11		✓	RIN N/A	
Sample 12		✓	RIN N/A	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/ μ l] : 150

Lower Marker Concentration [ng/ μ l] : 0

Upper Marker Concentration [ng/ μ l] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

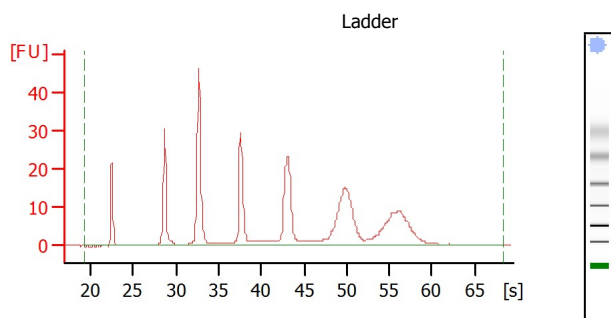
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

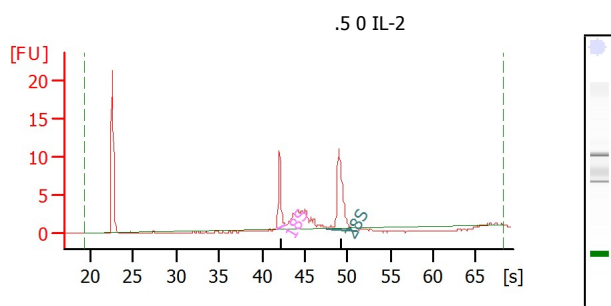
Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area: 364.5
RNA Concentration: 150 ng/μl
Result Flagging Color:
Result Flagging Label: All Other Samples



Overall Results for sample 1 : .5 0 IL-2

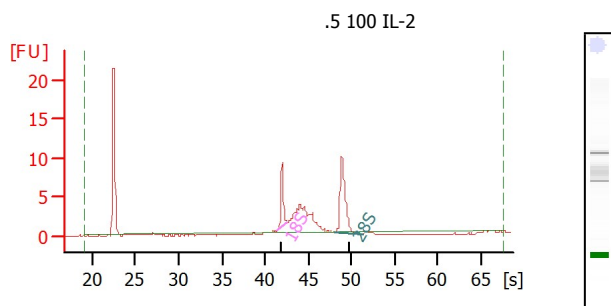
RNA Area: 38.3
RNA Concentration: 16 ng/μl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 9.8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.80

Fragment table for sample 1 : .5 0 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.69	42.77	7.6	20.0
28S	47.62	51.08	16.0	41.7

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

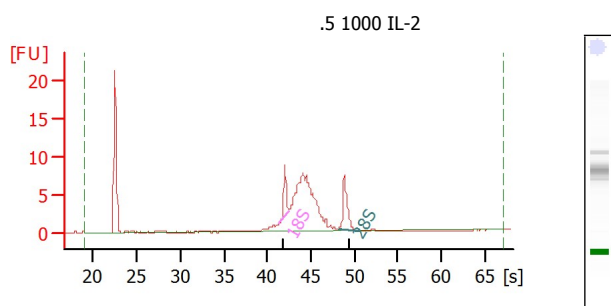
Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 2 : .5 100 IL-2**

RNA Area: 43.9
RNA Concentration: 18 ng/μl
rRNA Ratio [28s / 18s]: 2.2
RNA Integrity Number (RIN): 9.2 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.20

Fragment table for sample 2 : .5 100 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.32	42.65	6.3	14.4
28S	48.04	51.32	13.8	31.4

**Overall Results for sample 3 : .5 1000 IL-2**

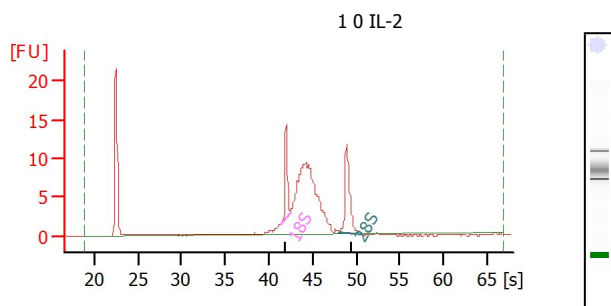
RNA Area: 66.3
RNA Concentration: 27 ng/μl
rRNA Ratio [28s / 18s]: 1.6
RNA Integrity Number (RIN): 7.1 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 7.10

Fragment table for sample 3 : .5 1000 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.40	42.47	5.2	7.8
28S	48.21	50.60	8.3	12.5

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

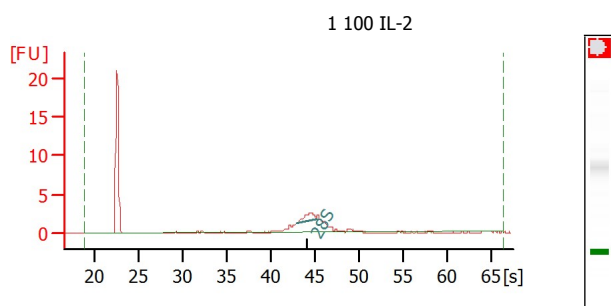
Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 4 : 1 0 IL-2**

RNA Area: 93.5
RNA Concentration: 38 ng/μl
rRNA Ratio [28s / 18s]: 1.6
RNA Integrity Number (RIN): 8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN:8

Fragment table for sample 4 : 1 0 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.46	42.53	8.5	9.1
28S	48.15	50.77	13.9	14.9

**Overall Results for sample 5 : 1 100 IL-2**

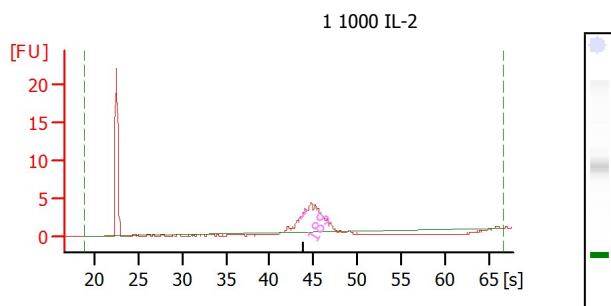
RNA Area: 24.4
RNA Concentration: 10 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 5 : 1 100 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
28S	43.02	45.19	2.9	12.0

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

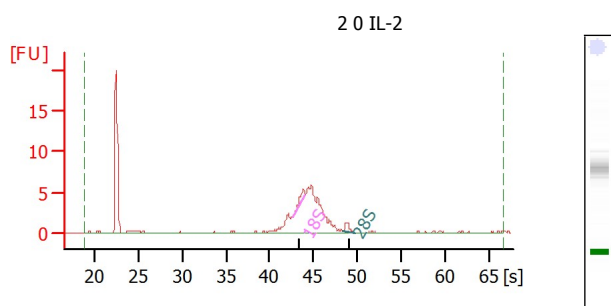
Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 6 : 1 1000 IL-2**

RNA Area: 24.5
RNA Concentration: 10 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): 2 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN:2

Fragment table for sample 6 : 1 1000 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.69	44.27	0.5	2.0

**Overall Results for sample 7 : 2 0 IL-2**

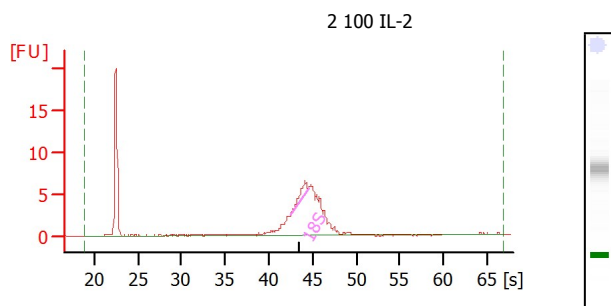
RNA Area: 46.9
RNA Concentration: 19 ng/μl
rRNA Ratio [28s / 18s]: 0.8
RNA Integrity Number (RIN): 2.8 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 2.80

Fragment table for sample 7 : 2 0 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.73	44.08	1.7	3.6
28S	48.44	49.84	1.4	3.0

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

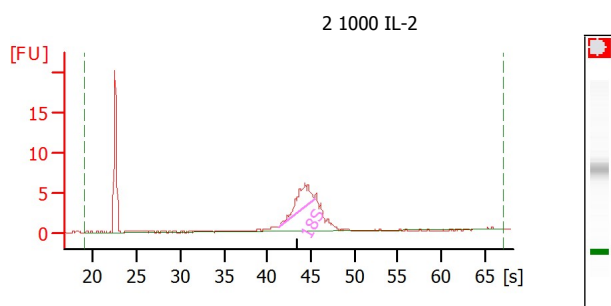
Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 8 : 2 100 IL-2**

RNA Area: 53.8
RNA Concentration: 22 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): 2.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 2.70

Fragment table for sample 8 : 2 100 IL-2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.58	44.52	2.8	5.3

**Overall Results for sample 9 : 2 1000 IL-2**

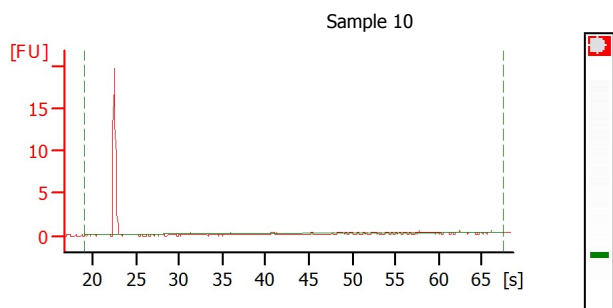
RNA Area: 47.6
RNA Concentration: 20 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 9 : 2 1000 IL-2

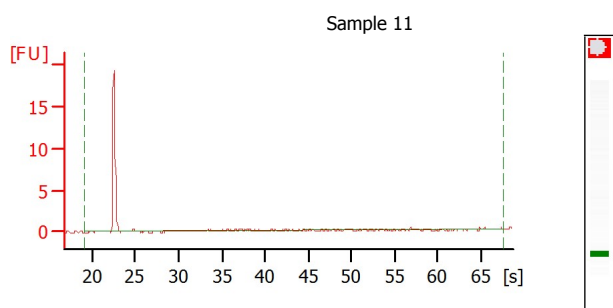
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.44	45.49	11.8	24.9

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 10 : Sample 10**

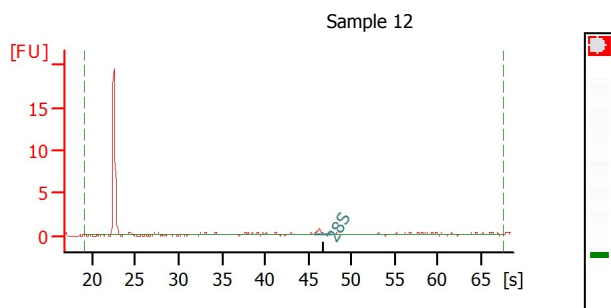
RNA Area: 2.9
RNA Concentration: 1 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

**Overall Results for sample 11 : Sample 11**

RNA Area: 3.9
RNA Concentration: 2 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Electropherogram Summary Continued ...**Overall Results for sample 12 : Sample 12**

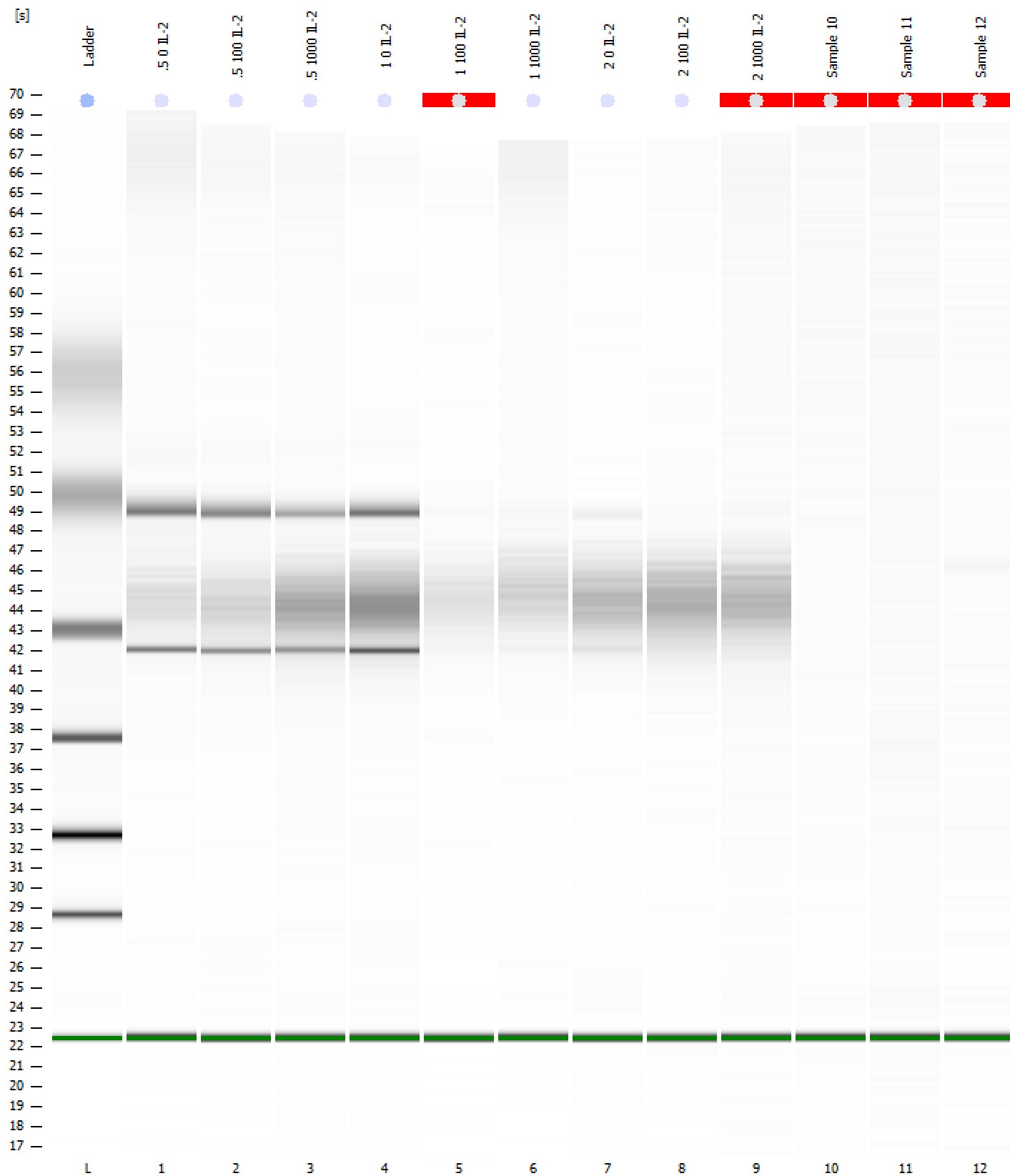
RNA Area: 5.9
RNA Concentration: 2 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 12 : Sample 12

Name	Start Time [s]	End Time [s]	Area	% of total Area
28S	45.74	47.60	1.0	16.3

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

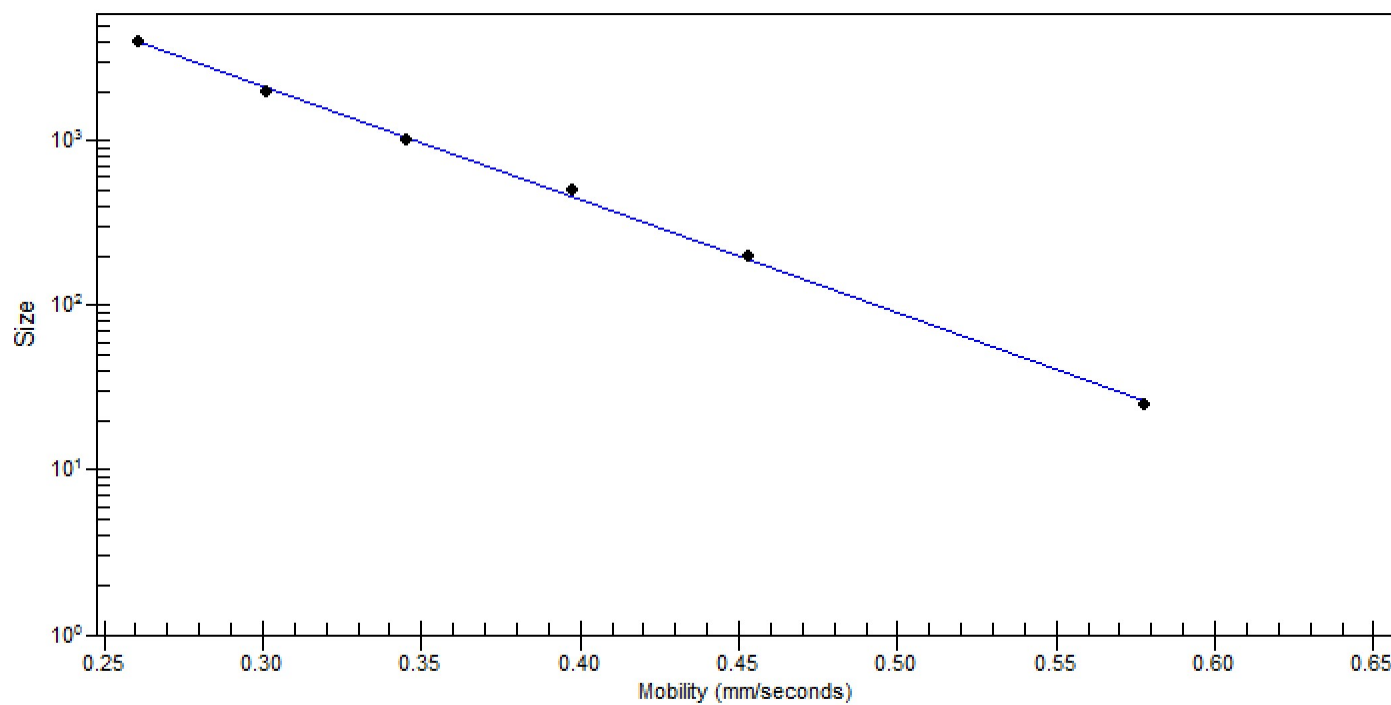
Gel Image

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

Created: 22/02/2020 6:36:48 PM
Modified: 22/02/2020 7:04:02 PM

Curves

Standard Curve



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad

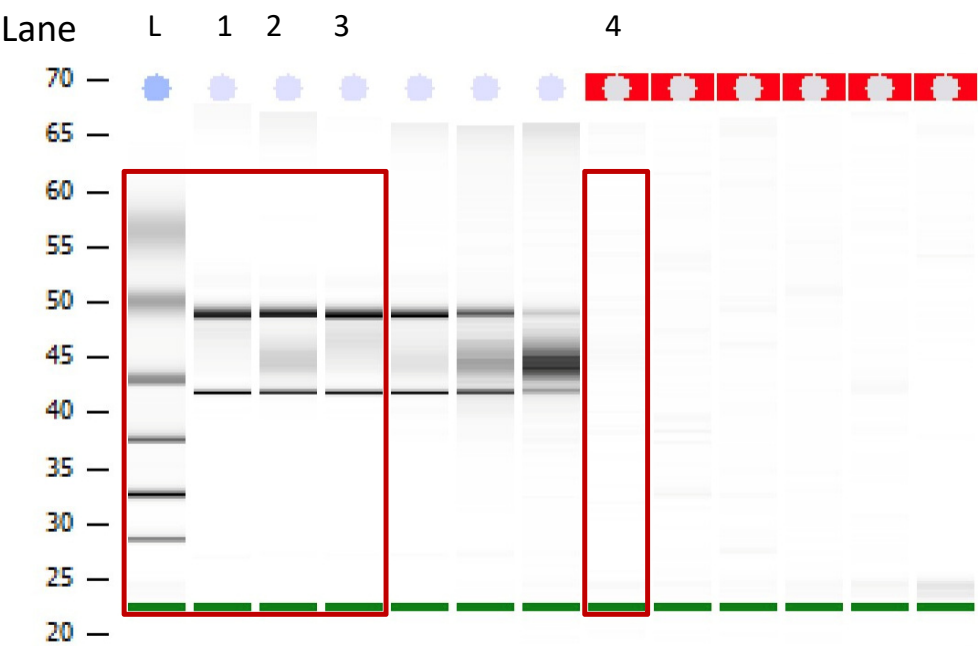
Created: 22/02/2020 6:36:48 PM
 Modified: 22/02/2020 7:04:02 PM

Run Logbook

Description	Number	Source	Category	Sub Category	Time	Time Zone	User	Host
Run ended on port 1 (Number of wells acquired: 13)		Instrument	Run		22/02/2020 6:59:51 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Run started on port 1 (File: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\Data\2020-02-22\2100 expert_Eukaryote Total RNA Nano_DEDAE00251_2020-02-22_18-36-48.xad)		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Product Number : G2939B		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Name :		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Vendor : Agilent Technologies		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Serial# : DEDAE00251		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Firmware : C.01.069		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH
Cartridge : Electrode		Instrument	Run		22/02/2020 6:36:53 PM	(GMT --05:00) Eastern Standard Time	ipascheto	HRSRH

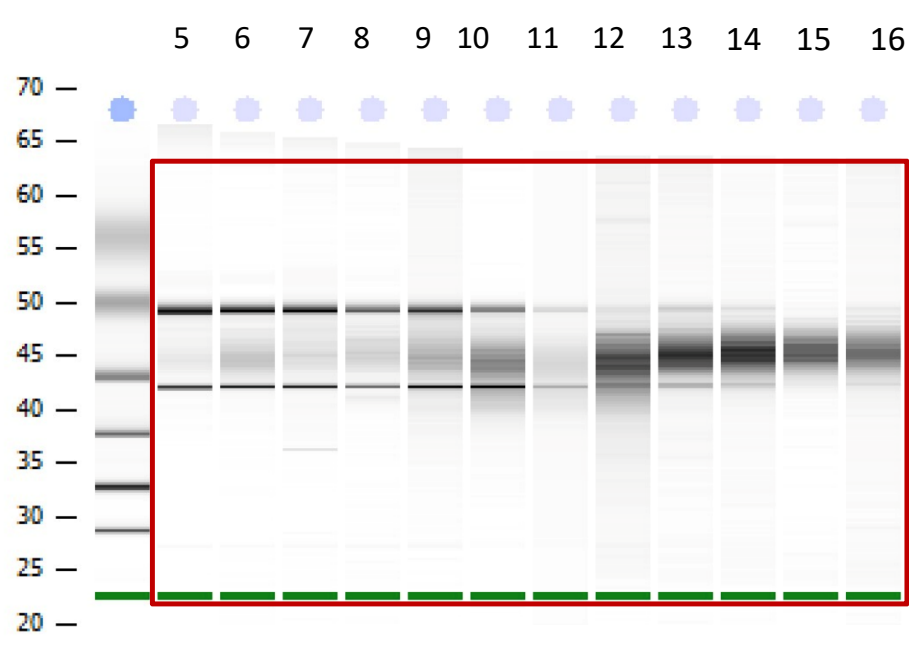
ORIGINAL DATA SUPPORTING COMPOSITE ELECTROPHEROGRAM IMAGES FOR FIGURE 5

Fig. 5. composite representative electropherogram



2100 Expert File Name: 2021-06-13_21-46-14

L = ladder

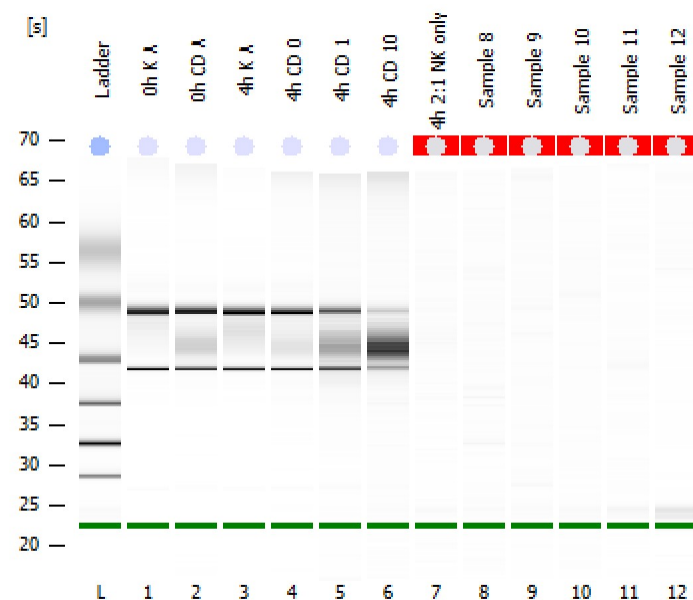


2100 Expert File Name: 2021-06-23_19-44-12

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DEDAE00251

Firmware: C.01.069

Serial#: DEDAE00251

Type: G2939B

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

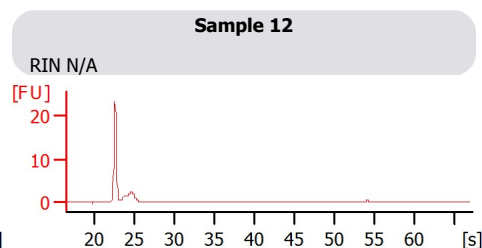
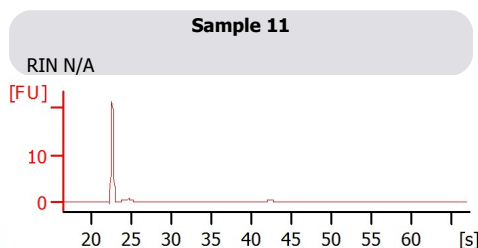
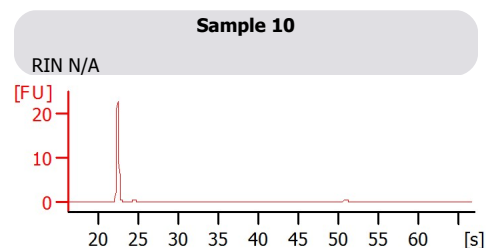
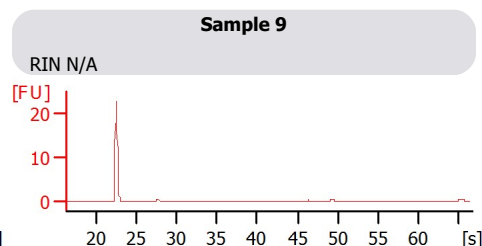
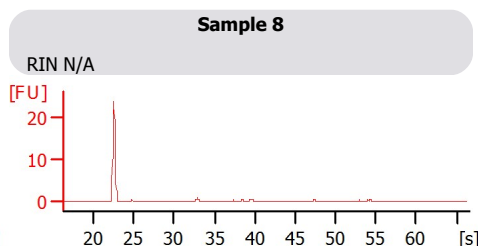
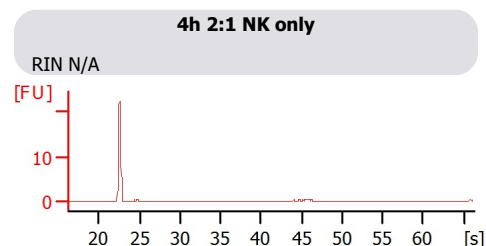
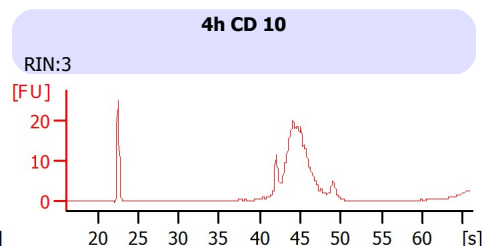
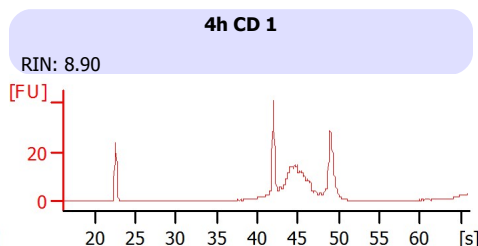
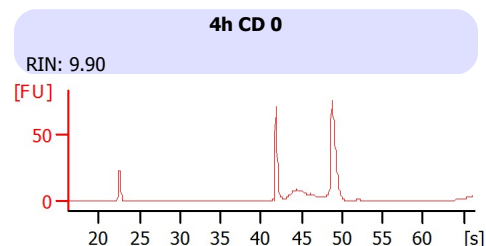
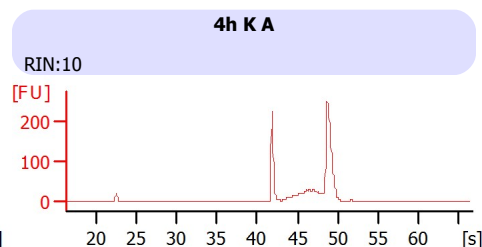
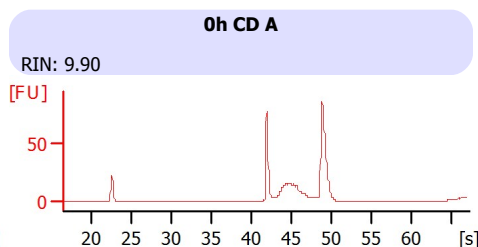
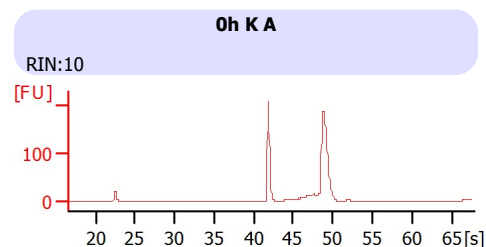
© Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments: Dec 10/2020 blood draw IM_09.



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
 Modified: 13/06/2021 10:25:55 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
0h K A		✓	RIN:10	
0h CD A		✓	RIN: 9.90	
4h K A		✓	RIN:10	
4h CD 0		✓	RIN: 9.90	
4h CD 1		✓	RIN: 8.90	
4h CD 10		✓	RIN:3	
4h 2:1 NK only		✓	RIN N/A	
Sample 8		✓	RIN N/A	
Sample 9		✓	RIN N/A	
Sample 10		✓	RIN N/A	
Sample 11		✓	RIN N/A	
Sample 12		✓	RIN N/A	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Dec 10/2020 blood draw IM_09.

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/μl] : 150

Lower Marker Concentration [ng/μl] : 0

Upper Marker Concentration [ng/μl] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

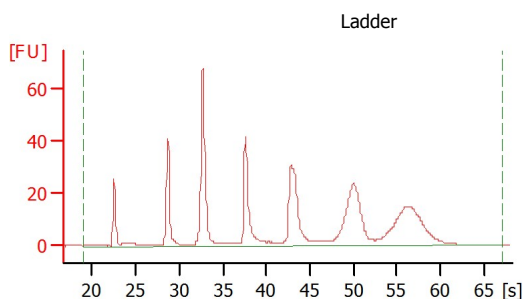
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

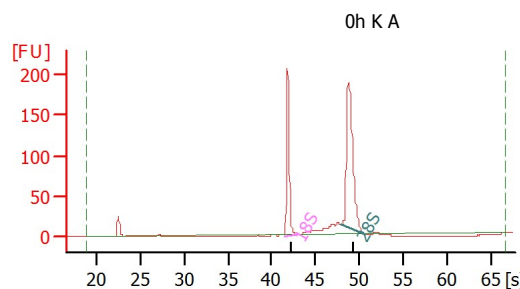
Created: 13/06/2021 9:46:14 PM
 Modified: 13/06/2021 10:25:55 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area: 571.0
 RNA Concentration: 150 ng/μl
 Result Flagging Color:
 Result Flagging Label: All Other Samples

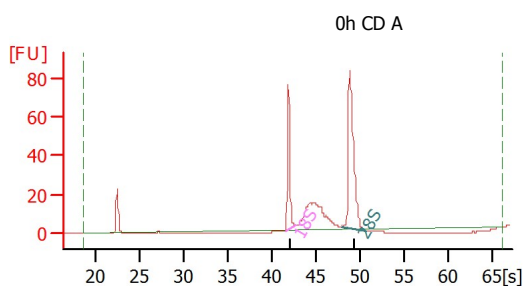


Overall Results for sample 1 : 0h K A

RNA Area: 572.0
 RNA Concentration: 150 ng/μl
 rRNA Ratio [28s / 18s]: 1.9
 RNA Integrity Number (RIN): 10 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN:10

Fragment table for sample 1 : 0h K A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.56	43.11	163.1	28.5
28S	48.05	50.66	304.2	53.2

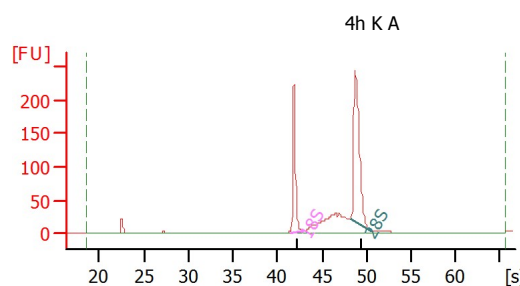


Overall Results for sample 2 : 0h CD A

RNA Area: 269.4
 RNA Concentration: 71 ng/μl
 rRNA Ratio [28s / 18s]: 2.0
 RNA Integrity Number (RIN): 9.9 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 9.90

Fragment table for sample 2 : 0h CD A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.59	42.84	58.1	21.6
28S	48.07	50.80	118.2	43.9



Overall Results for sample 3 : 4h K A

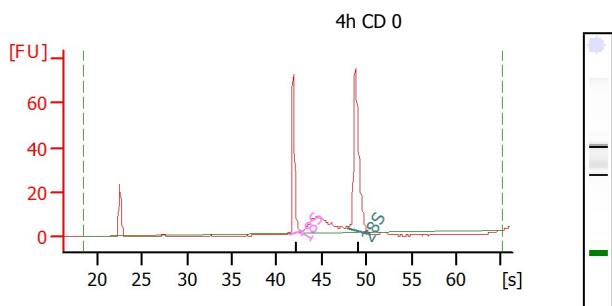
RNA Area: 816.7
 RNA Concentration: 215 ng/μl
 rRNA Ratio [28s / 18s]: 1.8
 RNA Integrity Number (RIN): 10 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN:10

Fragment table for sample 3 : 4h K A

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.53	42.91	186.2	22.8
28S	48.23	50.42	344.5	42.2

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

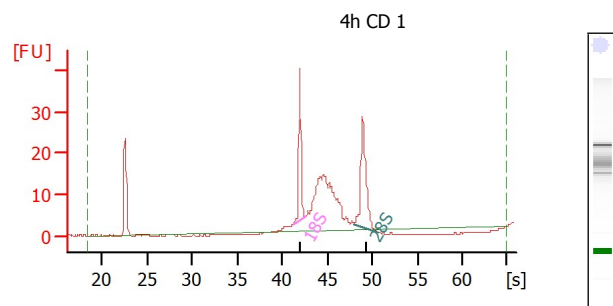
Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Electropherogram Summary Continued ...**Overall Results for sample 4 : 4h CD 0**

RNA Area: 200.7
RNA Concentration: 53 ng/μl
rRNA Ratio [28s / 18s]: 1.8
RNA Integrity Number (RIN): 9.9 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.90

Fragment table for sample 4 : 4h CD 0

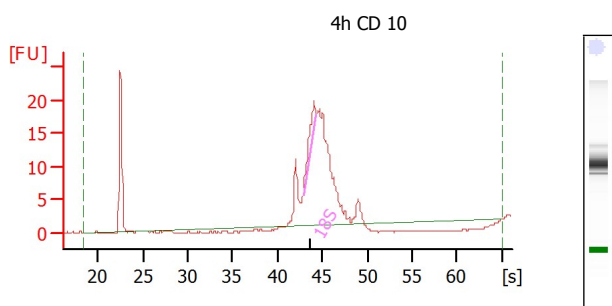
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.55	42.97	53.6	26.7
28S	47.93	50.48	97.4	48.5

**Overall Results for sample 5 : 4h CD 1**

RNA Area: 167.4
RNA Concentration: 44 ng/μl
rRNA Ratio [28s / 18s]: 1.4
RNA Integrity Number (RIN): 8.9 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.90

Fragment table for sample 5 : 4h CD 1

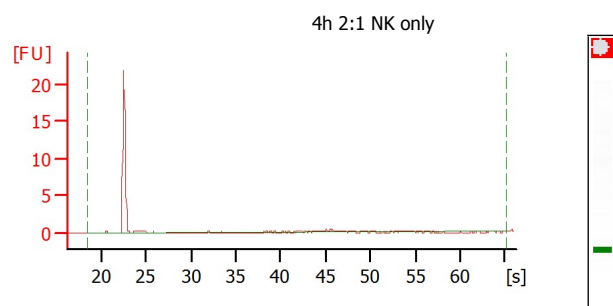
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.23	42.60	27.2	16.3
28S	47.92	50.65	37.8	22.6

**Overall Results for sample 6 : 4h CD 10**

RNA Area: 130.6
RNA Concentration: 34 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): 3 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 3

Fragment table for sample 6 : 4h CD 10

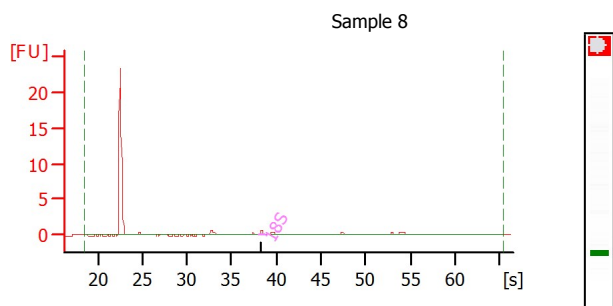
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.83	44.34	8.2	6.3

**Overall Results for sample 7**

RNA Area: 4.3
RNA Concentration: 1 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

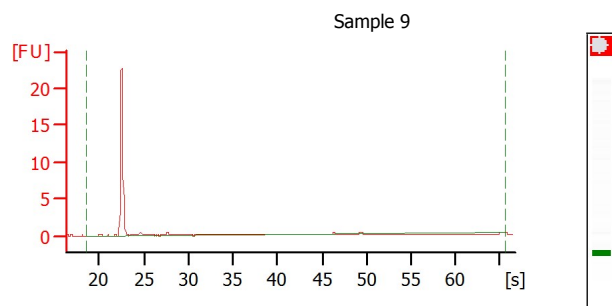
Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Electropherogram Summary Continued ...**Overall Results for sample 8 :** Sample 8

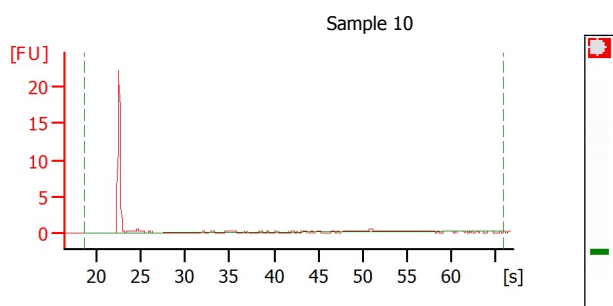
RNA Area: 2.9
RNA Concentration: 1 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 8 : Sample 8

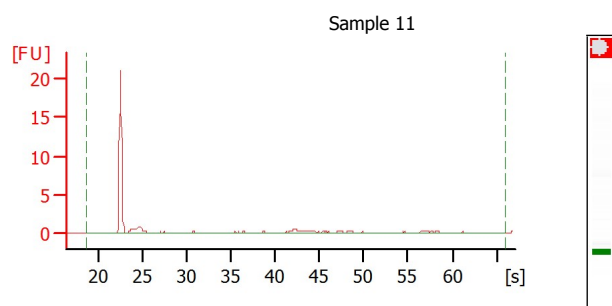
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	38.12	38.64	0.4	13.6

**Overall Results for sample 9 :** Sample 9

RNA Area: 2.5
RNA Concentration: 1 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

**Overall Results for sample 10 :** Sample 10

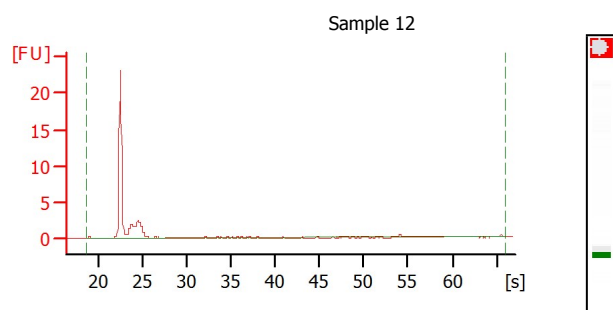
RNA Area: 6.1
RNA Concentration: 2 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

**Overall Results for sample 11 :** Sample 11

RNA Area: 11.6
RNA Concentration: 3 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

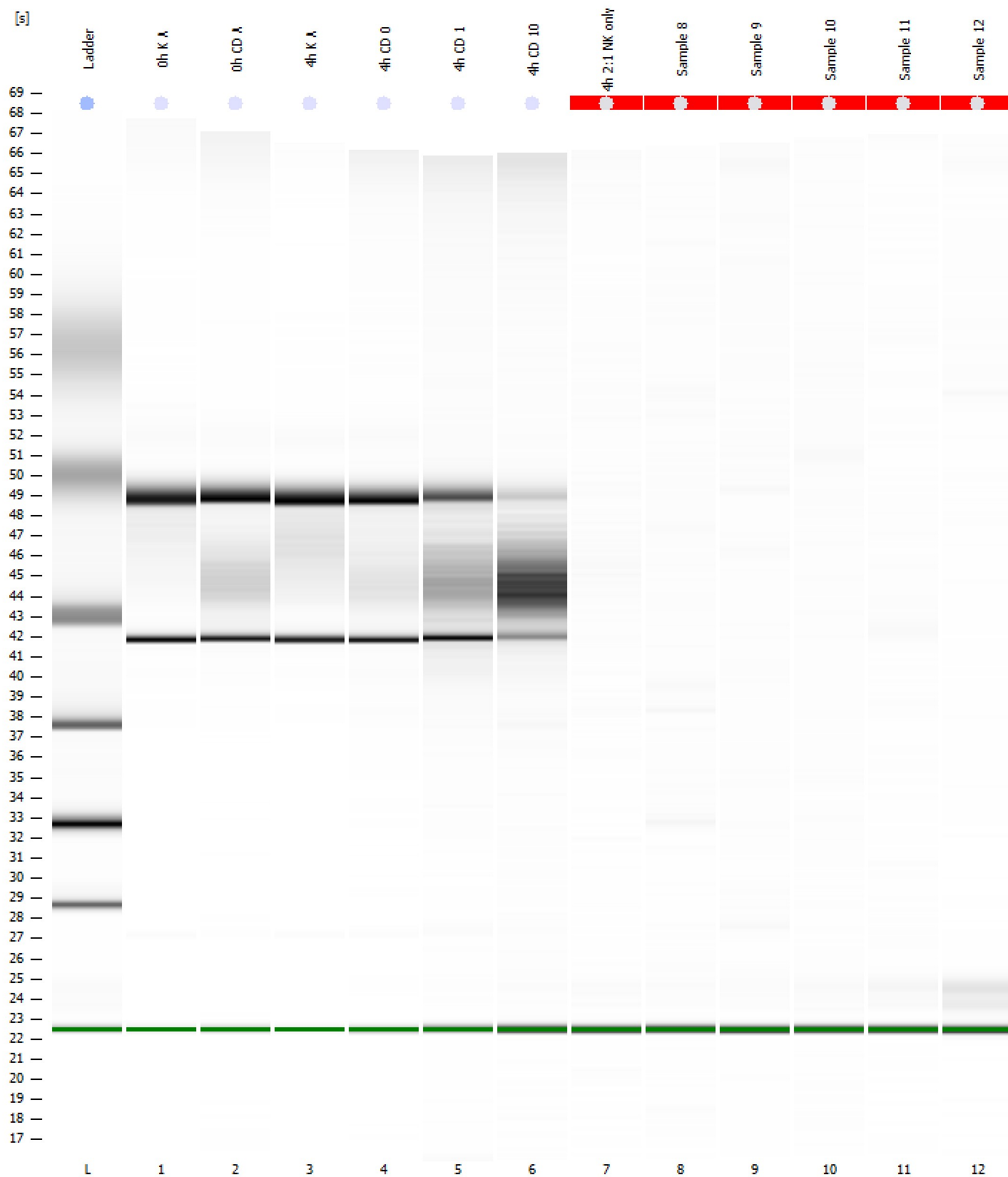
Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Electropherogram Summary Continued ...**Overall Results for sample 12 : Sample 12**

RNA Area: 14.6
RNA Concentration: 4 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

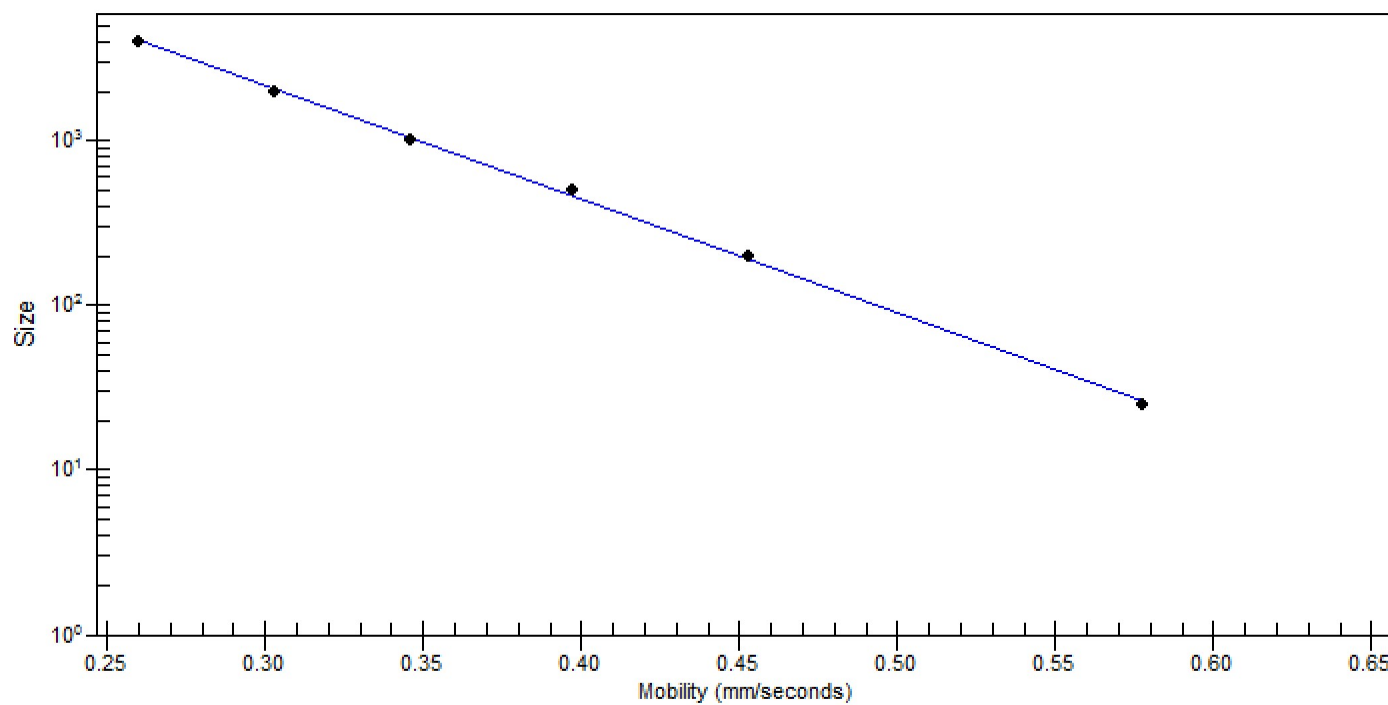
Gel Image

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
Modified: 13/06/2021 10:25:55 PM

Curves

Standard Curve



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad

Created: 13/06/2021 9:46:14 PM
 Modified: 13/06/2021 10:25:55 PM

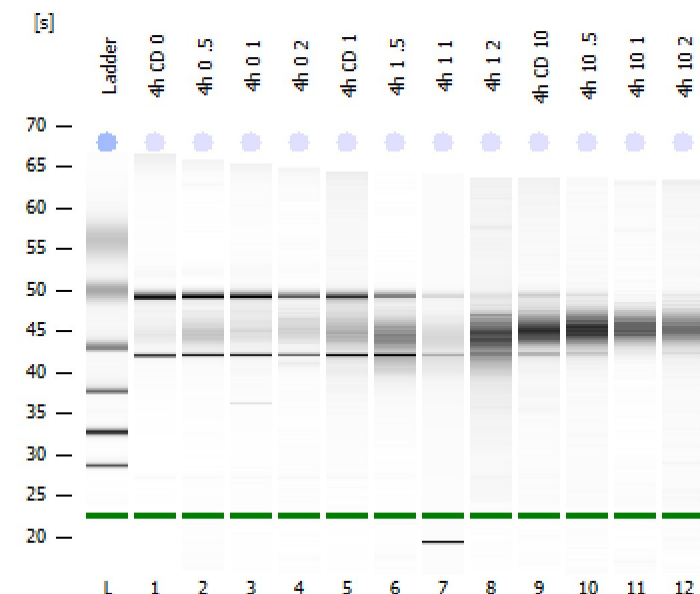
Run Logbook

Description	Number	Source	Category	Sub Category	Time	Time Zone	User	Host
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Run started on port 1 (File: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\Data\2021-06-13\2100 expert_Eukaryote Total RNA Nano_DEDAE00251_2021-06-13_21-46-14.xad)		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Product Number : G2939B		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Name :		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Vendor : Agilent Technologies		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Serial# : DEDAE00251		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Firmware : C.01.069		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Cartridge : Electrode		Instrument	Run		13/06/2021 9:46:21 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DEDAE00251
Serial#: DEDAE00251

Firmware: C.01.069
Type: G2939B

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

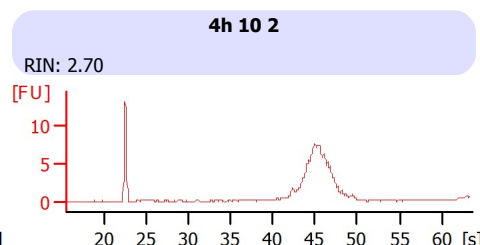
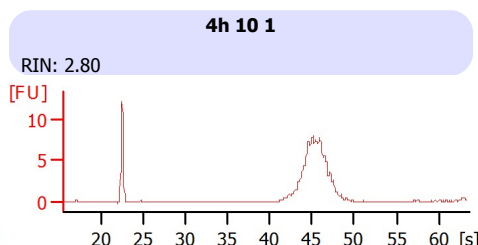
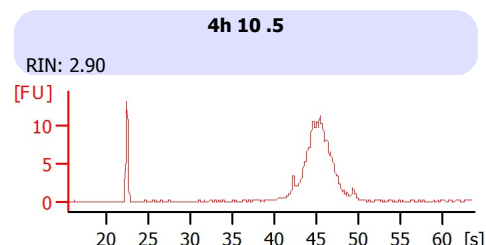
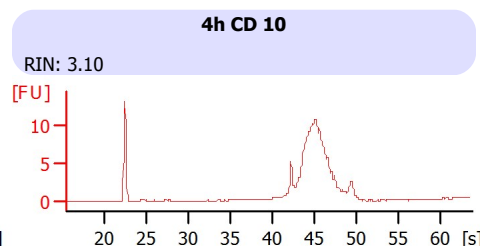
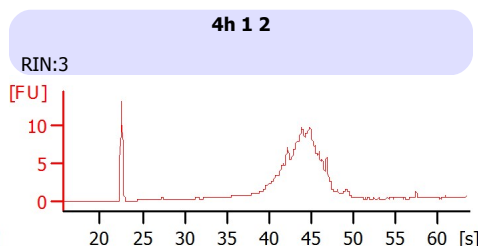
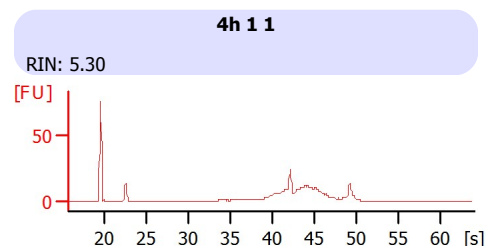
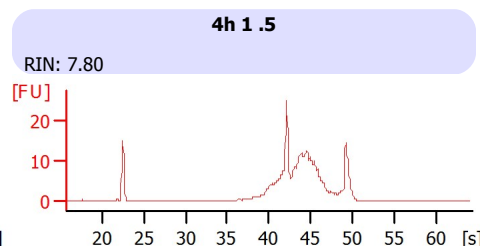
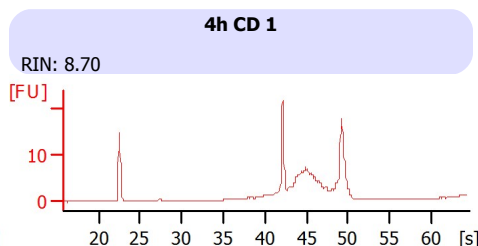
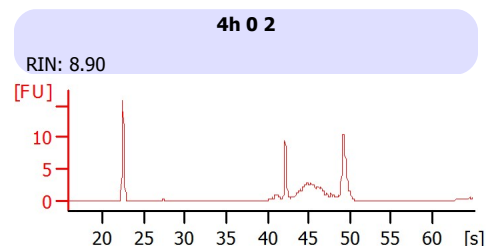
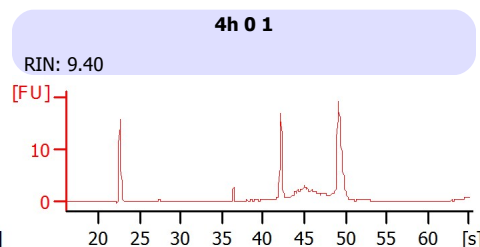
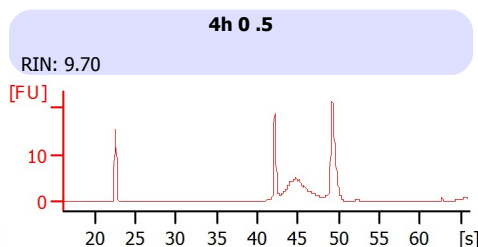
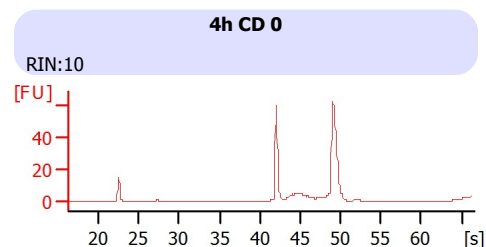
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Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments: Chip from December 10, 2020 blood draw of NK + DOX IM_09.



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
4h CD 0		✓	RIN:10	
4h 0 .5		✓	RIN: 9.70	
4h 0 1		✓	RIN: 9.40	
4h 0 2		✓	RIN: 8.90	
4h CD 1		✓	RIN: 8.70	
4h 1 .5		✓	RIN: 7.80	
4h 1 1		✓	RIN: 5.30	
4h 1 2		✓	RIN:3	
4h CD 10		✓	RIN: 3.10	
4h 10 .5		✓	RIN: 2.90	
4h 10 1		✓	RIN: 2.80	
4h 10 2		✓	RIN: 2.70	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Chip from December 10, 2020 blood draw of NK + DOX IM_09.

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/μl] : 150

Lower Marker Concentration [ng/μl] : 0

Upper Marker Concentration [ng/μl] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

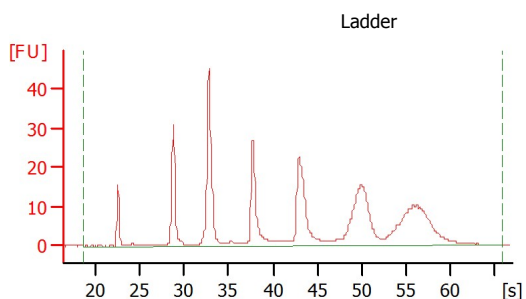
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

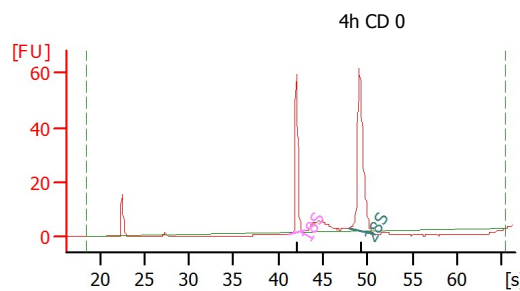
Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area: 401.0
RNA Concentration: 150 ng/μl
Result Flagging Color:
Result Flagging Label: All Other Samples

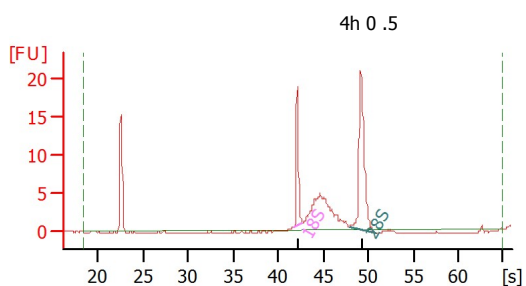


Overall Results for sample 1 : 4h CD 0

RNA Area: 156.3
RNA Concentration: 58 ng/μl
rRNA Ratio [28s / 18s]: 2.0
RNA Integrity Number (RIN): 10 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN:10

Fragment table for sample 1 : 4h CD 0

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.20	43.05	46.2	29.5
28S	47.90	50.89	90.3	57.8

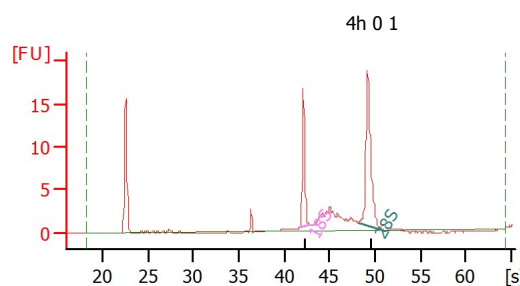


Overall Results for sample 2 : 4h 0.5

RNA Area: 72.0
RNA Concentration: 27 ng/μl
rRNA Ratio [28s / 18s]: 2.2
RNA Integrity Number (RIN): 9.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.70

Fragment table for sample 2 : 4h 0.5

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.66	42.69	13.0	18.1
28S	47.92	50.88	28.6	39.7



Overall Results for sample 3 : 4h 0 1

RNA Area: 58.1
RNA Concentration: 22 ng/μl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 9.4 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 9.40

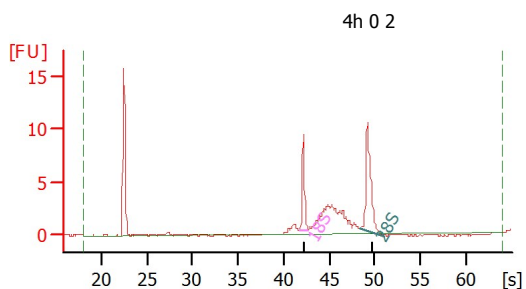
Fragment table for sample 3 : 4h 0 1

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.83	42.81	11.2	19.3
28S	48.13	51.12	23.3	40.1

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
 Modified: 23/06/2021 9:32:34 PM

Electropherogram Summary Continued ...

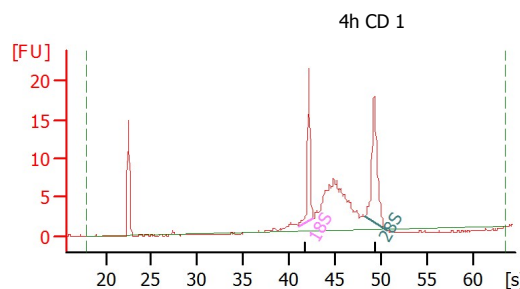


Overall Results for sample 4 : 4h 0 2

RNA Area: 41.0
 RNA Concentration: 15 ng/μl
 rRNA Ratio [28s / 18s]: 2.0
 RNA Integrity Number (RIN): 8.9 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.90

Fragment table for sample 4 : 4h 0 2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.80	42.73	6.3	15.4
28S	48.39	50.94	12.7	31.0

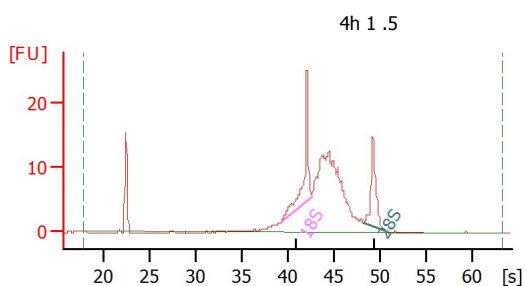


Overall Results for sample 5 : 4h CD 1

RNA Area: 96.3
 RNA Concentration: 36 ng/μl
 rRNA Ratio [28s / 18s]: 1.4
 RNA Integrity Number (RIN): 8.7 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.70

Fragment table for sample 5 : 4h CD 1

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.90	42.61	15.3	15.8
28S	48.22	50.52	22.0	22.9

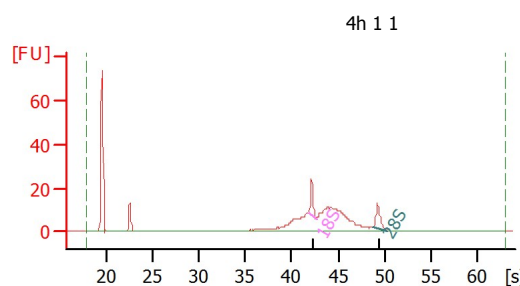


Overall Results for sample 6 : 4h 1.5

RNA Area: 171.2
 RNA Concentration: 64 ng/μl
 rRNA Ratio [28s / 18s]: 0.8
 RNA Integrity Number (RIN): 7.8 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 7.80

Fragment table for sample 6 : 4h 1.5

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	39.26	42.66	23.8	13.9
28S	48.21	50.69	17.8	10.4



Overall Results for sample 7 : 4h 1 1

RNA Area: 274.0
 RNA Concentration: 103 ng/μl
 rRNA Ratio [28s / 18s]: 1.0
 RNA Integrity Number (RIN): 5.3 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 5.30

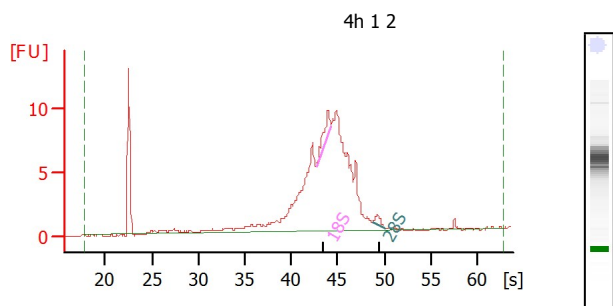
Fragment table for sample 7 : 4h 1 1

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.84	42.62	11.1	4.1
28S	48.75	50.35	10.6	3.9

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
 Modified: 23/06/2021 9:32:34 PM

Electropherogram Summary Continued ...

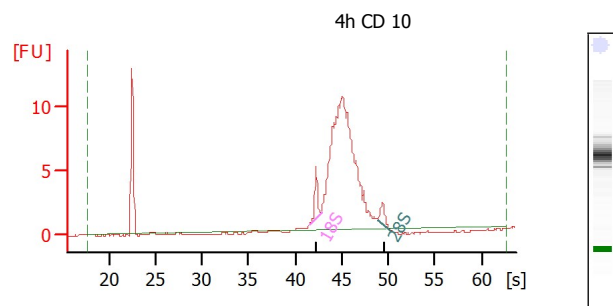


Overall Results for sample 8 : 4h 1 2

RNA Area: 118.6
 RNA Concentration: 44 ng/μl
 rRNA Ratio [28s / 18s]: 0.3
 RNA Integrity Number (RIN): 3 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN:3

Fragment table for sample 8 : 4h 1 2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.77	44.32	3.8	3.2
28S	48.69	50.10	1.1	0.9

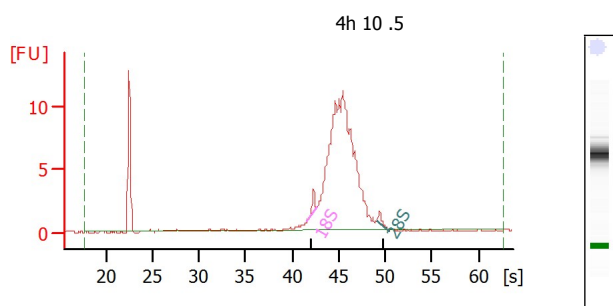


Overall Results for sample 9 : 4h CD 10

RNA Area: 78.0
 RNA Concentration: 29 ng/μl
 rRNA Ratio [28s / 18s]: 0.6
 RNA Integrity Number (RIN): 3.1 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 3.10

Fragment table for sample 9 : 4h CD 10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.55	42.77	3.1	4.0
28S	48.91	50.14	1.8	2.4

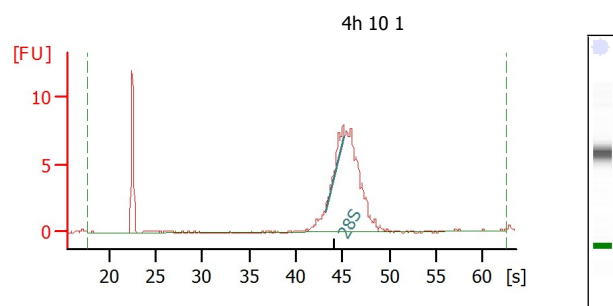


Overall Results for sample 10 : 4h 10 .5

RNA Area: 88.6
 RNA Concentration: 33 ng/μl
 rRNA Ratio [28s / 18s]: 0.6
 RNA Integrity Number (RIN): 2.9 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 2.90

Fragment table for sample 10 : 4h 10 .5

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.64	42.68	1.5	1.6
28S	49.05	50.45	0.9	1.0



Overall Results for sample 11 : 4h 10 1

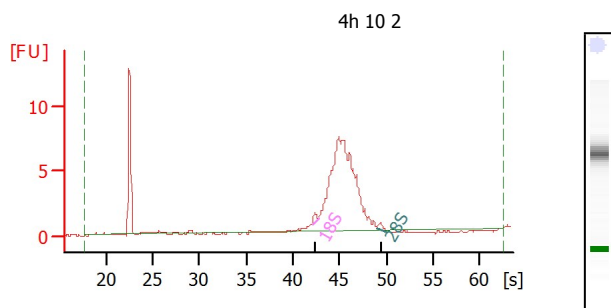
RNA Area: 56.0
 RNA Concentration: 21 ng/μl
 rRNA Ratio [28s / 18s]: 0.0
 RNA Integrity Number (RIN): 2.8 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 2.80

Fragment table for sample 11 : 4h 10 1

Name	Start Time [s]	End Time [s]	Area	% of total Area
28S	43.19	45.23	3.3	5.8

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Electropherogram Summary Continued ...**Overall Results for sample 12 : 4h 10 2**

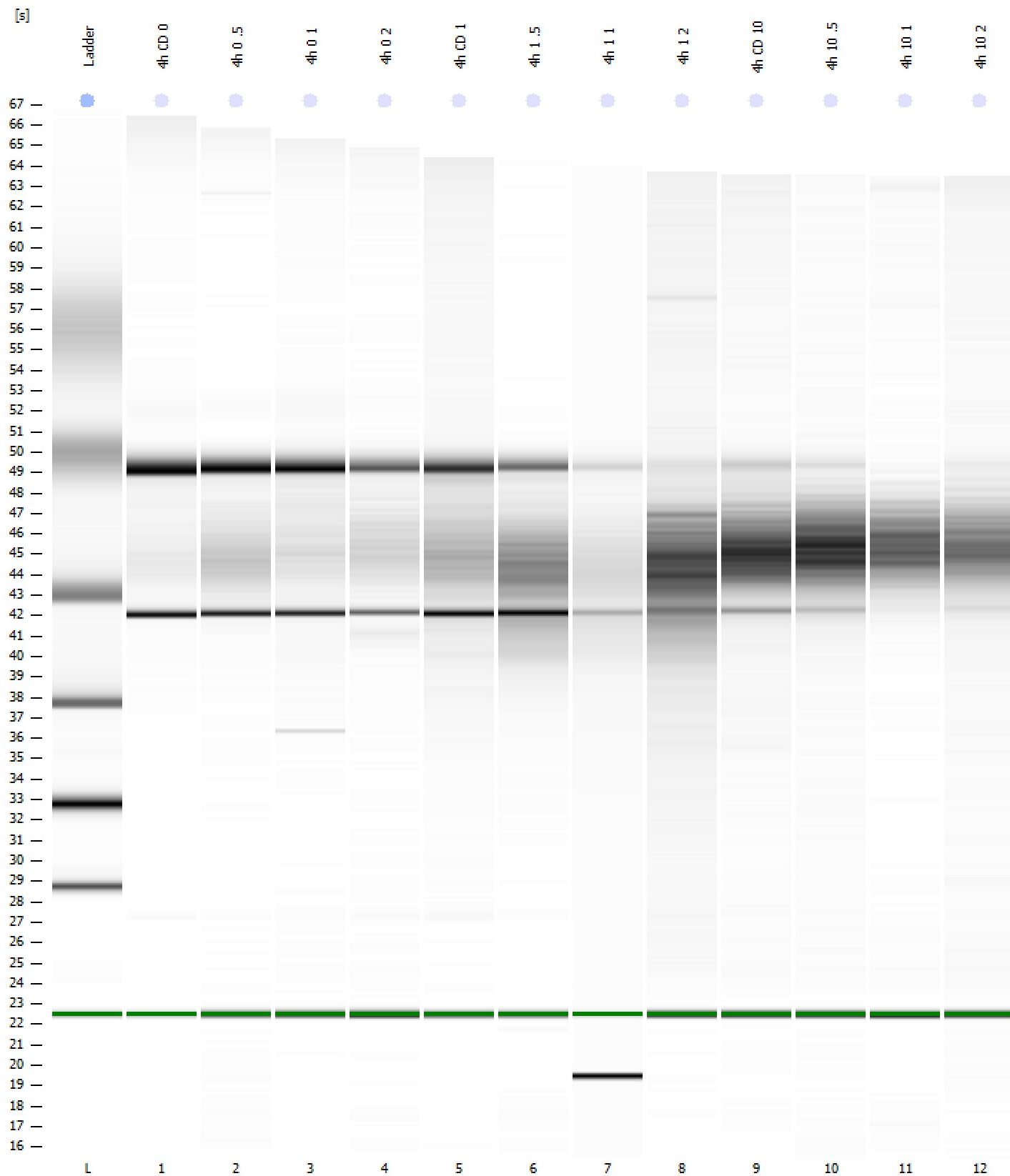
RNA Area: 56.9
RNA Concentration: 21 ng/μl
rRNA Ratio [28s / 18s]: 1.0
RNA Integrity Number (RIN): 2.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 2.70

Fragment table for sample 12 : 4h 10 2

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.05	42.69	0.5	0.9
28S	48.99	50.13	0.5	0.9

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

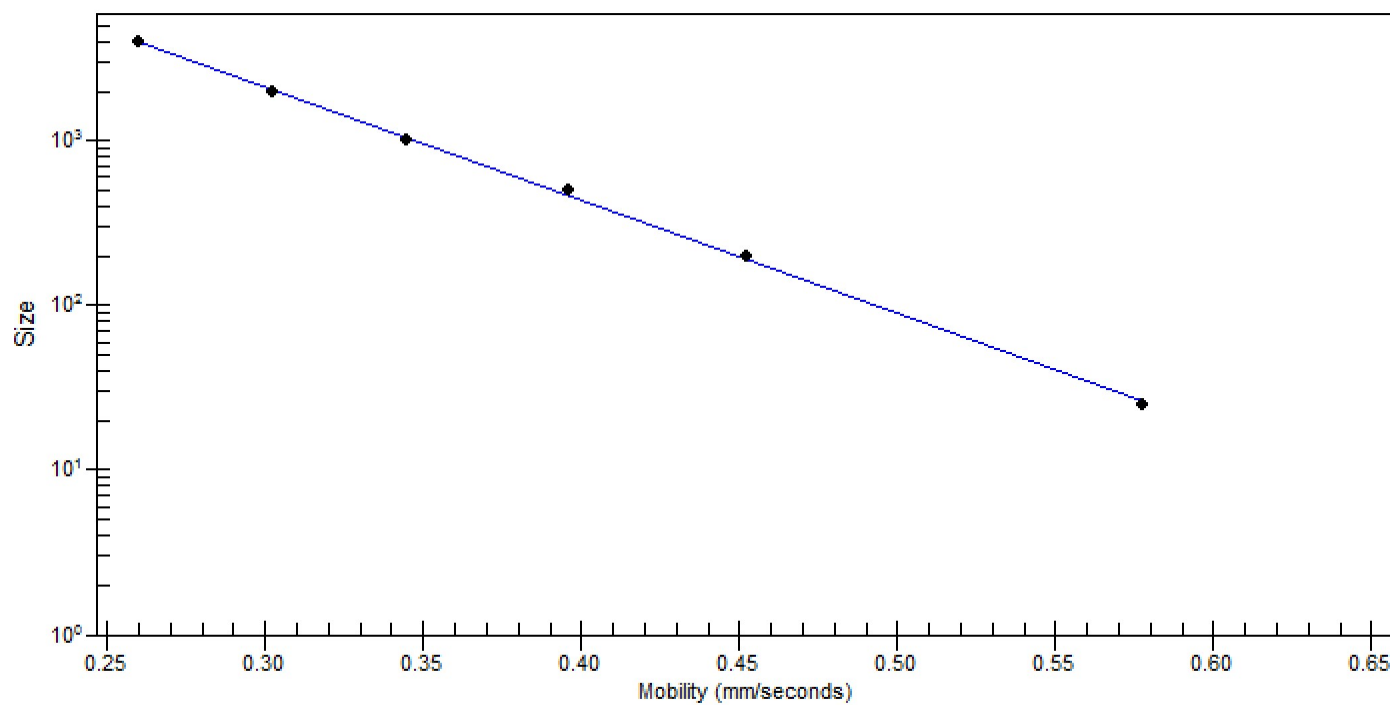
Gel Image

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

Created: 23/06/2021 7:44:11 PM
Modified: 23/06/2021 9:32:34 PM

Curves

Standard Curve



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad

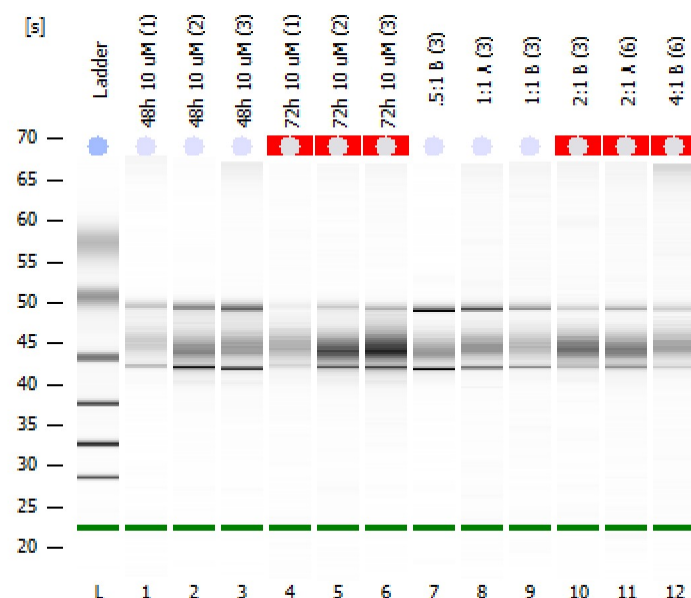
Created: 23/06/2021 7:44:11 PM
 Modified: 23/06/2021 9:32:34 PM

Run Logbook

Description	Number	Source	Category	Sub Category	Time	Time Zone	User	Host
Run ended on port 1 (Number of wells acquired: 13)		Instrument	Run		23/06/2021 8:08:01 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Run started on port 1 (File: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\Data\2021-06-23\2100 expert_Eukaryote Total RNA Nano_DEDAE00251_2021-06-23_19-44-12.xad)		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Product Number : G2939B		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Name :		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Vendor : Agilent Technologies		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Serial# : DEDAE00251		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Firmware : C.01.069		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Cartridge : Electrode		Instrument	Run		23/06/2021 7:44:17 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Electrophoresis File Run SummaryInstrument Information:

Instrument Name: DEDAE00251

Firmware: C.01.069

Serial#: DEDAE00251

Type: G2939B

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

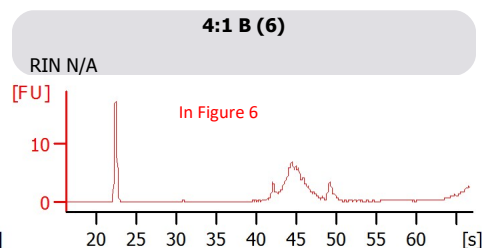
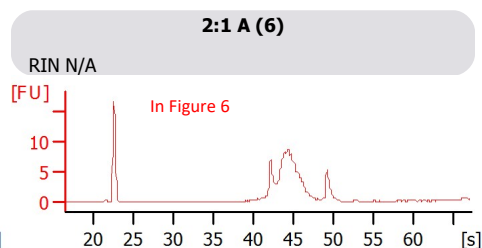
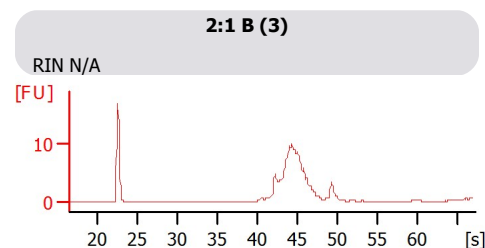
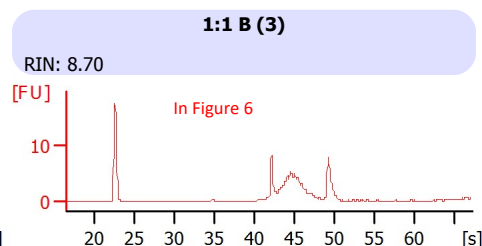
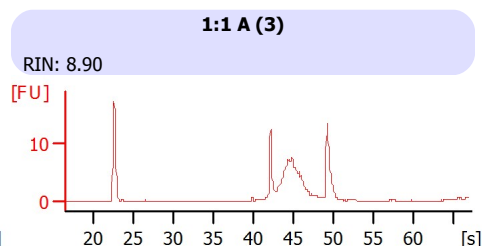
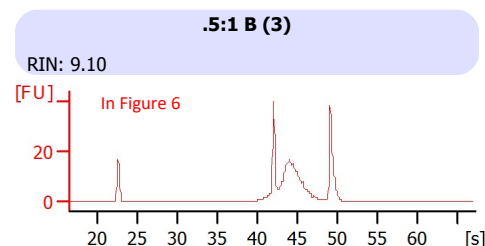
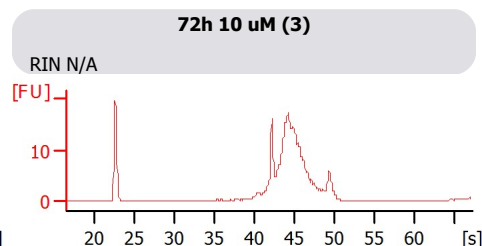
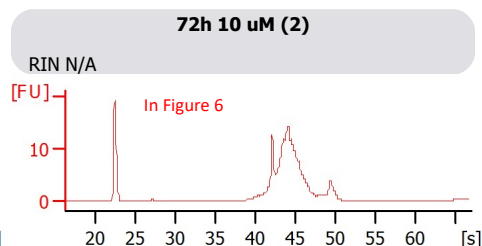
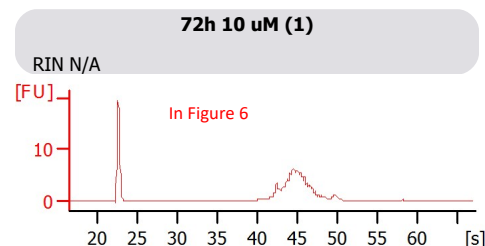
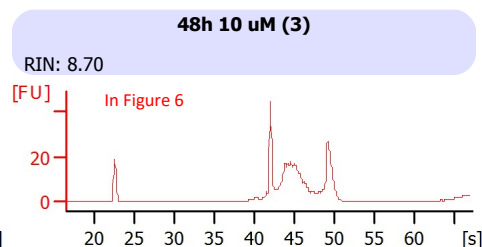
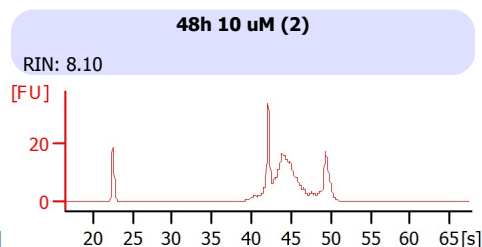
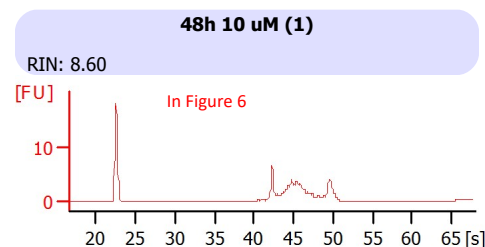
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Chip Information:

Chip Lot #:

Reagent Kit Lot #:

Chip Comments:



Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
48h 10 uM (1)		✓	RIN: 8.60	
48h 10 uM (2)		✓	RIN: 8.10	
48h 10 uM (3)		✓	RIN: 8.70	
72h 10 uM (1)		✓	RIN N/A	
72h 10 uM (2)		✓	RIN N/A	
72h 10 uM (3)		✓	RIN N/A	
.5:1 B (3)		✓	RIN: 9.10	
1:1 A (3)		✓	RIN: 8.90	
1:1 B (3)		✓	RIN: 8.70	
2:1 B (3)		✓	RIN N/A	
2:1 A (6)		✓	RIN N/A	
4:1 B (6)		✓	RIN N/A	
Ladder		✓	All Other Samples	

Chip Lot #**Reagent Kit Lot #****Chip Comments :**

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Electrophoresis Assay Details

General Analysis Settings

Number of Available Sample and Ladder Wells (Max.) : 13

Minimum Visible Range [s] : 17

Maximum Visible Range [s] : 70

Start Analysis Time Range [s] : 19

End Analysis Time Range [s] : 69

Ladder Concentration [ng/ μ l] : 150

Lower Marker Concentration [ng/ μ l] : 0

Upper Marker Concentration [ng/ μ l] : 0

Used Lower Marker for Quantitation

Standard Curve Fit is Logarithmic

Show Data Aligned to Lower Marker

Integrator Settings

Integration Start Time [s] : 19

Integration End Time [s] : 69

Slope Threshold : 0.6

Height Threshold [FU] : 0.5

Area Threshold : 0.2

Width Threshold [s] : 0.5

Baseline Plateau [s] : 6

Filter Settings

Filter Width [s] : 0.5

Polynomial Order : 4

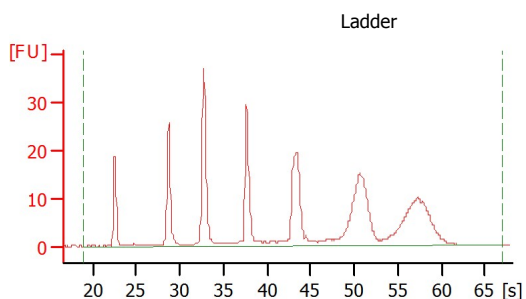
Ladder

Ladder Peak	Size
1	25
2	200
3	500
4	1000
5	2000
6	4000

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

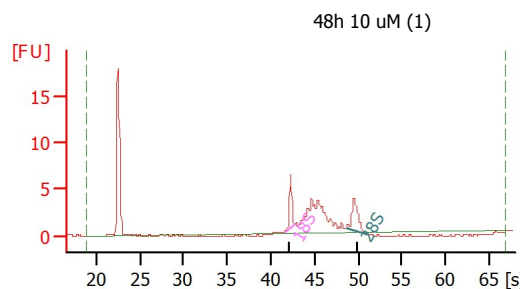
Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area: 345.3
RNA Concentration: 150 ng/μl
Result Flagging Color:
Result Flagging Label: All Other Samples

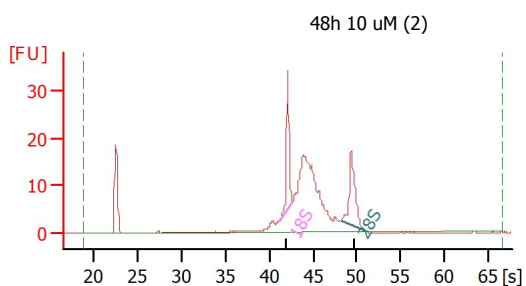


Overall Results for sample 1 : 48h 10 uM (1)

RNA Area: 35.5
RNA Concentration: 15 ng/μl
rRNA Ratio [28s / 18s]: 1.4
RNA Integrity Number (RIN): 8.6 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.60

Fragment table for sample 1 : 48h 10 uM (1)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.51	42.77	4.5	12.7
28S	48.64	51.06	6.1	17.2

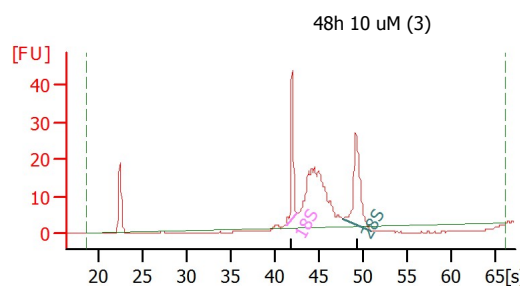


Overall Results for sample 2 : 48h 10 uM (2)

RNA Area: 194.0
RNA Concentration: 84 ng/μl
rRNA Ratio [28s / 18s]: 1.0
RNA Integrity Number (RIN): 8.1 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.10

Fragment table for sample 2 : 48h 10 uM (2)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.14	42.68	24.9	12.8
28S	48.19	51.13	25.1	13.0



Overall Results for sample 3 : 48h 10 uM (3)

RNA Area: 193.7
RNA Concentration: 84 ng/μl
rRNA Ratio [28s / 18s]: 1.3
RNA Integrity Number (RIN): 8.7 (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN: 8.70

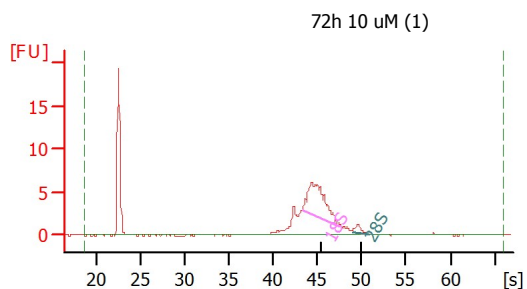
Fragment table for sample 3 : 48h 10 uM (3)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.31	42.51	31.8	16.4
28S	47.83	50.90	40.8	21.1

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
 Modified: 02/08/2021 8:46:27 PM

Electropherogram Summary Continued ...

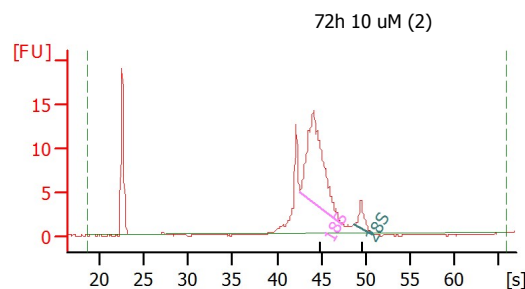


Overall Results for sample 4 : 72h 10 uM (1)

RNA Area: 52.6
 RNA Concentration: 23 ng/μl
 rRNA Ratio [28s / 18s]: 0.1
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

Fragment table for sample 4 : 72h 10 uM (1)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	43.28	47.35	19.9	37.8
28S	48.97	50.84	1.5	2.9

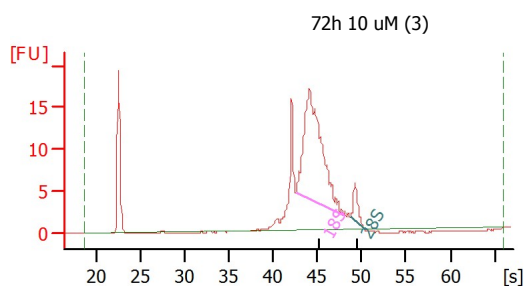


Overall Results for sample 5 : 72h 10 uM (2)

RNA Area: 107.8
 RNA Concentration: 47 ng/μl
 rRNA Ratio [28s / 18s]: 0.1
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

Fragment table for sample 5 : 72h 10 uM (2)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.56	47.25	47.3	43.9
28S	48.77	50.78	4.1	3.8

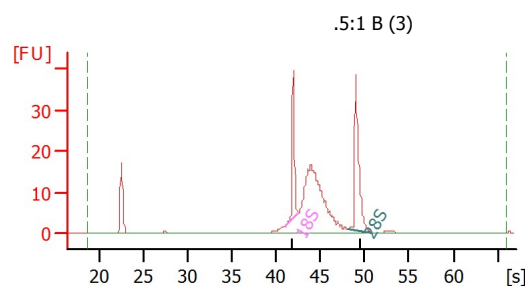


Overall Results for sample 6 : 72h 10 uM (3)

RNA Area: 140.5
 RNA Concentration: 61 ng/μl
 rRNA Ratio [28s / 18s]: 0.1
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

Fragment table for sample 6 : 72h 10 uM (3)

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.56	47.87	68.8	49.0
28S	48.73	50.27	5.2	3.7



Overall Results for sample 7

RNA Area: 182.3
 RNA Concentration: 79 ng/μl
 rRNA Ratio [28s / 18s]: 1.5
 RNA Integrity Number (RIN): 9.1 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 9.10

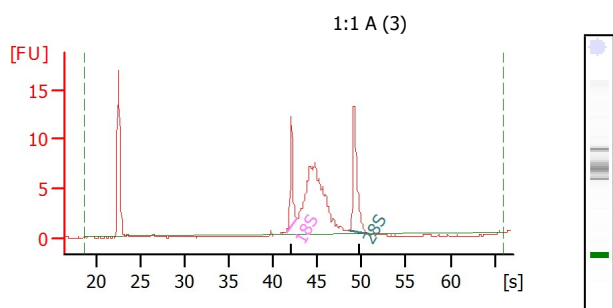
Fragment table for sample 7

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.12	42.46	26.8	14.7
28S	48.21	50.84	40.7	22.3

Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
 Modified: 02/08/2021 8:46:27 PM

Electropherogram Summary Continued ...

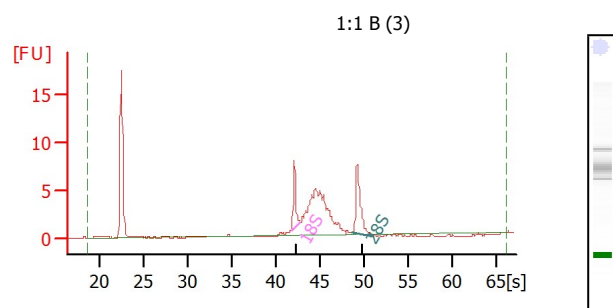


Overall Results for sample 8

RNA Area: 67.1
 RNA Concentration: 29 ng/μl
 rRNA Ratio [28s / 18s]: 1.6
 RNA Integrity Number (RIN): 8.9 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.90

Fragment table for sample 8

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.46	42.65	8.4	12.6
28S	48.64	51.13	13.7	20.5

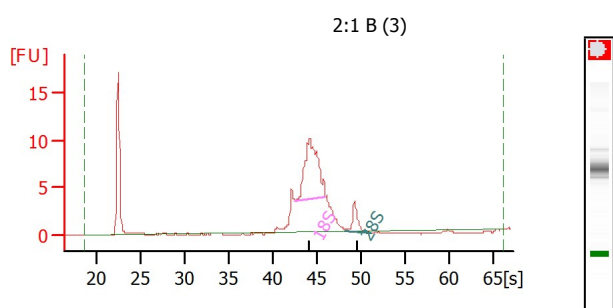


Overall Results for sample 9

RNA Area: 46.8
 RNA Concentration: 20 ng/μl
 rRNA Ratio [28s / 18s]: 1.5
 RNA Integrity Number (RIN): 8.7 (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN: 8.70

Fragment table for sample 9

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.74	42.79	5.5	11.8
28S	48.69	50.85	8.5	18.1

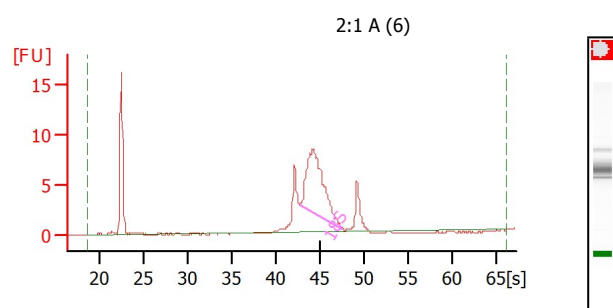


Overall Results for sample 10

RNA Area: 71.5
 RNA Concentration: 31 ng/μl
 rRNA Ratio [28s / 18s]: 0.2
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

Fragment table for sample 10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.55	46.01	24.3	34.0
28S	48.21	51.14	4.4	6.2



Overall Results for sample 11

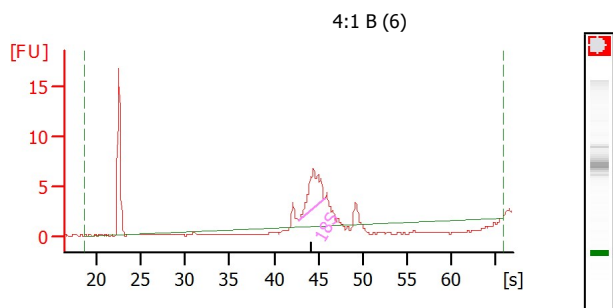
RNA Area: 67.7
 RNA Concentration: 29 ng/μl
 rRNA Ratio [28s / 18s]: 0.0
 RNA Integrity Number (RIN): N/A (B.02.10)
 Result Flagging Color:
 Result Flagging Label: RIN N/A

Fragment table for sample 11

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.70	47.45	31.5	46.6

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Electropherogram Summary Continued ...**Overall Results for sample 12**

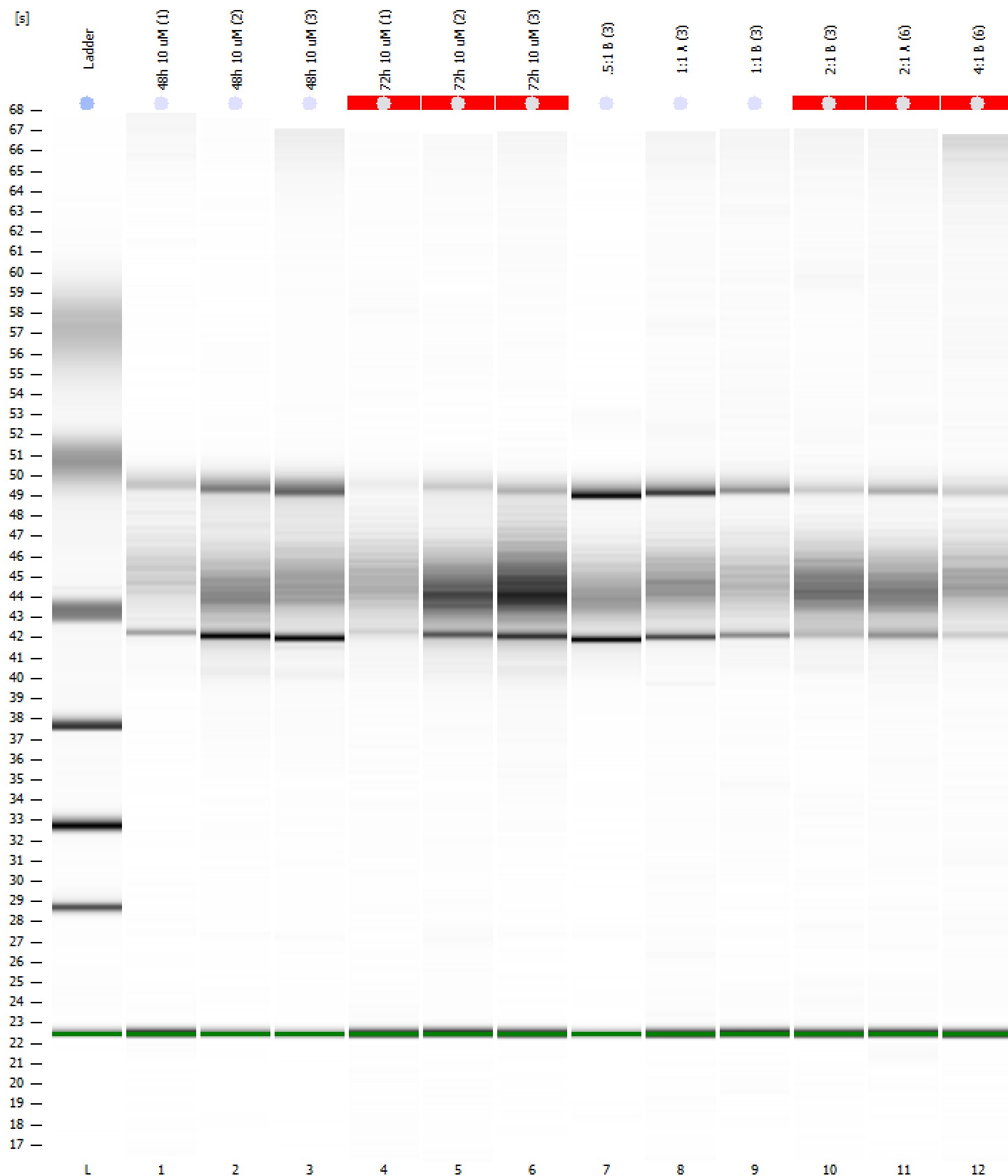
RNA Area: 34.2
RNA Concentration: 15 ng/μl
rRNA Ratio [28s / 18s]: 0.0
RNA Integrity Number (RIN): N/A (B.02.10)
Result Flagging Color:
Result Flagging Label: RIN N/A

Fragment table for sample 12

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.80	45.76	12.4	36.1

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

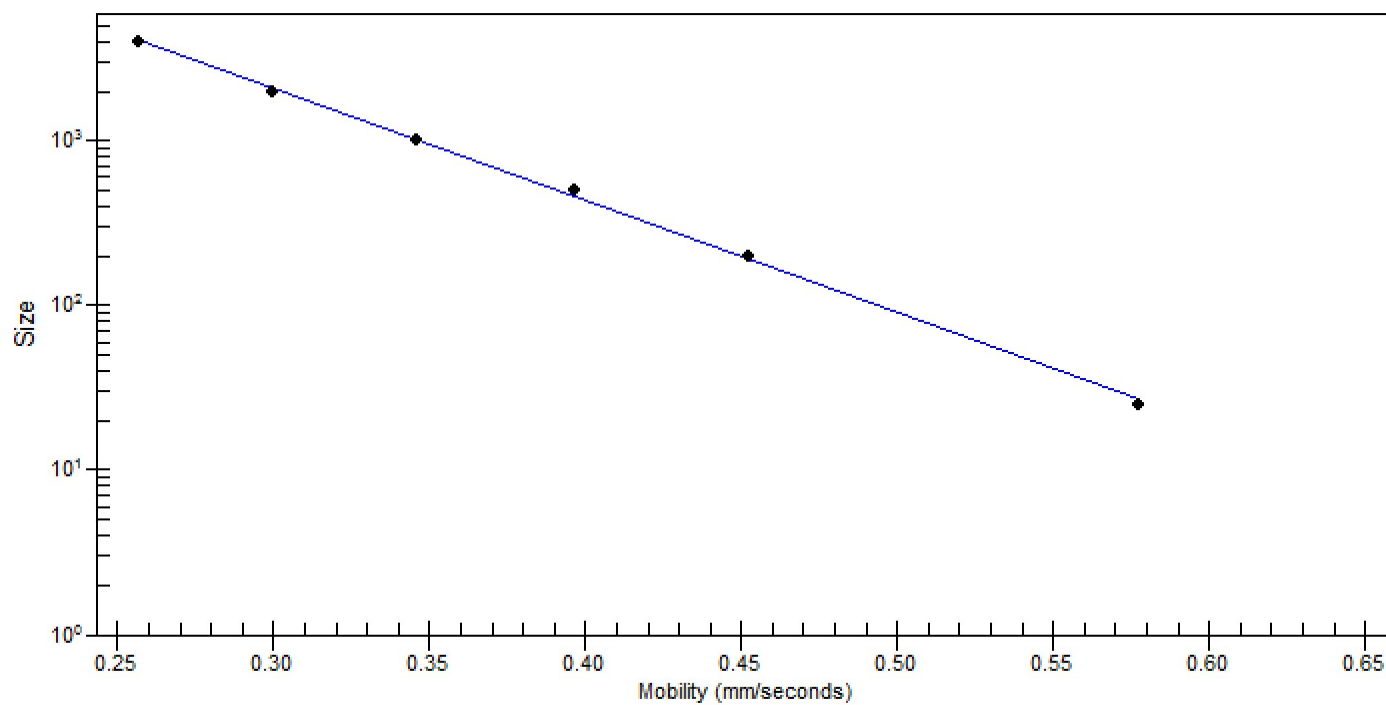
Gel Image

Assay Class: Eukaryote Total RNA Nano
Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
Modified: 02/08/2021 8:46:27 PM

Curves

Standard Curve



Assay Class: Eukaryote Total RNA Nano
 Data Path: K:\...Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad

Created: 02/08/2021 7:47:51 PM
 Modified: 02/08/2021 8:46:27 PM

Run Logbook

Description	Number	Source	Category	Sub Category	Time	Time Zone	User	Host
Run ended on port 1 (Number of wells acquired: 13)		Instrument	Run		02/08/2021 8:10:50 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Run started on port 1 (File: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\Data\2021-08-02\2100 expert_Eukaryote Total RNA Nano_DEDAE00251_2021-08-02_19-47-52.xad)		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Product Number : G2939B		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Name :		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Vendor : Agilent Technologies		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Serial# : DEDAE00251		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Firmware : C.01.069		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH
Cartridge : Electrode		Instrument	Run		02/08/2021 7:47:52 PM	(GMT --04:00) Eastern Standard Time	ipascheto	HRSRH