

Certificate of Analysis

C O N F I D E N T I A L

Antibody Full Length Sequencing of Hybridoma 7C8F1

Order Number: U4466HJ110-5

Report From:

GenScript USA Inc

860 Centennial Ave

Piscataway, NJ 08854

United States of America

For research use only

This study was conducted by strictly following the procedures described in this report. All data presented are authentic and accurate to the best of our knowledge. Information and materials provided by this investigation are for research use only.

Recommended Analysis Tools

We recommend the following free online tools for sequence analysis of immunoglobulin variable regions:

- i. NCBI Nucleotide BLAST
- ii. IMGT/V Quest program
- iii. NCBI IgBLAST

Antibody sequences of 7C8F1 were listed as below:

Heavy chain: DNA sequence (1398 bp)

Signal sequence-**FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon**

ATGAACTTAGGGCTCAGCTTCATTTTCCTTGCCCTTATTTTAAAAGGTGTCCAGTGT**GAGGTGCAGC**
TGGTGGAGTCTGGGGGAGGCTTCGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGG
ATTCACTTTCACTCGCTATGGCATGTCTTGGTTTCGCCAGACTCCAGACAAGAGGCTGGAGTTGGTC
GCAAACATTAATGGTAATGGTGGTAGCACCTATTATTCAGACAGTGTGAAGGGCCGATTCACCATTT
CTAGAGACAATGCCAAGAACACCCTGTTCTACAAATGAGCAGTCTGAAGTCTGAGGACGCAGCCAT
GTATTCCTGTGCAAGAGATCGGGGCTACTATTGGTACGAAGGCGCTATGGACTACTGGGGTCAAGGA
ACCTCAGTCACCGTCTCCTCAGCCAAAACGACACCCCATCTGTCTATCCACTGGCCCCTGGATCTG
CTGCCCAAATAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGAC
AGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGAC
CTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCA
ACGTTGCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGTGGTTGTAA
GCCTTGCAATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGCCCAAGGATGTG
CTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGG
TCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCA
GTTCAACAGCACTTTCCGCTCAGTCAGTGAACCTCCCATCATGCACCAGGACTGGCTCAATGGCAAG
GAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCTGCCCCCATCGAGAAAACCATCTCCAAAACCA
AAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCACCTCCCAAGGAGCAGATGGCCAAGGATAA
AGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGAAT
GGGCAGCCAGCGGAGAACTACAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCT
ACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTACCTGCTCTGTGTTACA
TGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGA

Heavy chain: Amino acid sequence (465 aa)

Signal peptide-**FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon**

MNLGLSFIFLALILKGVQC**EVQLVESGGGFVQPGGSLKLS****CAASGFTFSRYGMSWFRQTPDKRLELV**
ANINGNGGSTYYSDSVKGRFTISRDAKNTLFLQMSS**LKSEDAAMYSCARDRGYYWYEGAMDYWGQG**

TSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
LYTLSSSVTVPSSTWPSSETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDV
LTITLTPKVTCVVVDISKDDPEVQFSWFVDDDEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGK
EFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWN
GQPAENYKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK-

Light chain: DNA sequence (717 bp)

Signal sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCTGCTTCCAGCAGTGATGTTGTGA
TGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAG
TCAGAGCCTTGTACACAGTAATGGAAACACCTATTTACATTGGTATCTGCAGAAGCCAGGCCAGTCT
CCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTTCAAGTGGCAGTG
GATCAGGGACAGATTTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTCTG
CTCTCAAAGTACACATGTTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTGAT
GCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
TGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACG
ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGC
ACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGA
CATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTAG

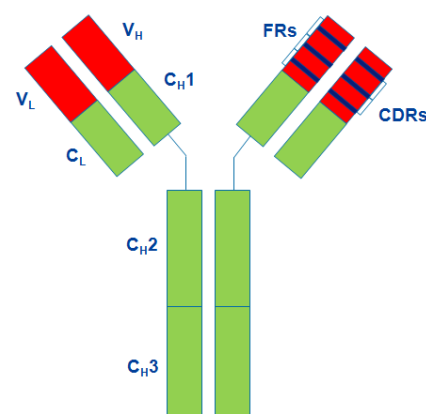
Light chain: Amino acid sequence (238 aa)

Signal peptide-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

MKLPVRLLVLMFWIPASSSDVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQS
PKLLIYKVSNRFSGVDPDRFSGSGTDFTLKISRVEAEDLGVYFCQSSTHVPWTFGGGTKLEIKRAD
AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVWKIDGSRQNGVLNSWTDQDSKDSTYSMS
SLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC-

Project Specifications

Sequence	Clones sequence	Clones with >99% sequence identity
V _H	≥5	≥5
V _L	≥5	≥5
C _H	≥5	≥5
C _L	≥5	≥5



IMGT Analysis of V(D)J Junctions

Sequence	V-GENE and allele	Functionality	V-REGION identity % (nt)	J-GENE and allele	D-GENE and allele	AA Junction	Junction frame
V _H	Musmus IGHV5-6*01	productive	95.9% (284/296 nt)	Musmus IGHJ4*01	Musmus IGHD2-14*01	CARDRG YYWYEG AMDYW	in-frame
V _L	Musmus IGKV1-110*01	productive	99.7% (298/299 nt)	Musmus IGKJ1*01	-	CSQSTH VPWTF	in-frame

Isotype of antibody

Isotype is Mouse IgG1/kappa, as analyzed by the sequences of constant region.

Materials

Hybridoma cells prepared by GenScript;

SMARTScribe Reverse Transcriptase (TaKaRa, Cat. No.: 639536);

RNA-easy Isolation Reagent (Vazyme, Cat. No. : R701-01-AA).

Methods

Total RNA was isolated from the hybridoma cells following the technical manual of RNA-easy Isolation Reagent. Total RNA was then reverse transcribed into cDNA using isotype-specific anti-sense primers or universal primers following the technical manual of SMARTScribe Reverse Transcriptase. The antibody fragments of V_H and V_L were amplified according to the standard operating procedure (SOP) of rapid amplification of cDNA ends (RACE) of GenScript. Amplified antibody fragments were cloned into a standard cloning vector separately. Colony PCR was performed to screen for clones with inserts of correct sizes. No less than five colonies with inserts of correct sizes were sequenced for each fragment. The sequences of different clones were aligned and the consensus sequence of these clones was

provided.

Date: 11/2/2022

Approved by

Bingying Luo

Luo Bingying

Antibody Department

GenScript USA Inc.