

Certificate of Analysis

CONFIDENTIAL

Antibody Full Length Sequencing of Hybridoma 4B3B5

Order Number: U4466HJ110-9

Report From:

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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 4B3B5 were listed as below:

Heavy chain: DNA sequence (1383 bp)

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

ATGGAAAGGCACTGGATCTTTCTCTTCCTGTTTTTCAGTAACTGCAGGTGTCCACTCC**CAGGTCCAGC**
TTCAGCAATCTGGGGCTGAACTGGCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGG
CTACACCTTTACTAGCTACTGGATGCAC**TGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGATT**
GGATACATTAATCCTGTC**ACTGGTTATACTGAATACAATCAGAACTTCAAGGAC****AAGGCCACATTGA**
CTGCAGACAAATCCTCCAGCACAGCCTTCATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGT
CTATTACTGTGCAAGAGGGGGGGGGGTACGGATCTTCTAC**TGGGGCCAAGGCACCACTCTCACAGTC**
TCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCCTGGATCTGCTGCCCAA**ACTAACT**
CCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGA**ACTC**
TGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCTGCAGTCTGACCTCTACACTCTGAGC
AGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCACCCGG
CCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTAC
AGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTG
ACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGT
TTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTT
CCGCTCAGTCAGTGA**ACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGG**
GTCAACAGTGCAGCTTTCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAGGCAGACCGAAGG
CTCCACAGGTGTACACCATTCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTG
CATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGAATGGGCAGCCAGCGGAG
AACTACAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATG
TGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTACCTGCTCTGTGTTACATGAGGGCCTGCACAA
CCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGA

Heavy chain: Amino acid sequence (460aa)

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

MERHWIFLFLFSVTAGVHS**QVQLQQSGAELAKPGASVKMSCKASGYTFTSYWMH****WVKQRPQGQGLEWI**
GYINPVTGYTEYNQNFKD**KATLTADKSSSTAFMQLS****SLTSEDSAVYYCARGGGVRI****IFYWGQGTTLTV**

SSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLS
SSVTVPSSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITL
TPKVTCVVVDISKDDPEVQFSWFVDDDEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR
VNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAE
NYKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK-

Light chain: DNA sequence (717 bp)

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

ATGGAGAAAGACACACTCCTGCTATGGGTCCCTGCTTCTCTGGGTTCAGGTTCACAGGTGACATTG
TGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCAGAGC
CAGCGAAAGTGTTGATAATTATGGCATTAGTTTTATGAAC TGGTTCCAACAGAAACCAGGACAGCCA
CCCAAACCTCCTCATCTATGCTGCATCCAACCAAGGATCCGGGGTCCCTGCCAGGTTTAGTGCCAGTG
GGTCTGGGACAGACTTCAGCCTCAACATCCATCCTATGGAGGAGGATGATACTGCAATGTATTTCTG
TCAGCAAAGTAAGGAGGTTCCCTTGGACGTTCCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTGAT
GCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
TGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACG
ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGC
ACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGA
CATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT TAG

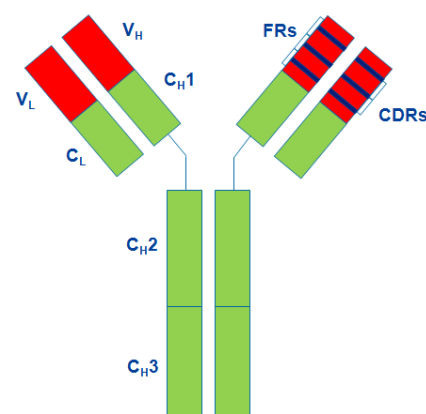
Light chain: Amino acid sequence (238 aa)

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

MEKDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQKPGQP
PKLLIYAASNQGSVGPARGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKRAD
AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVWKIDGSERQNGVLNSWTDQDSKDSTYSMSS
TLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC-

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 IGHV1-7*01	productive	95.9% (282/294 nt)	Musmus IGHJ2*01	Musmus IGHD2-14*01	CARGGG VRIFYW	in-frame
V _L	Musmus IGKV3-2*01	productive	100% (297/297 nt)	Musmus IGKJ1*01	-	CQQSKE 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: 11/3/2022

Approved by

Bingying Luo

Luo Bingying

Antibody Department

GenScript USA Inc.