

Certificate of Analysis

CONFIDENTIAL

Antibody Full Length Sequencing of Hybridoma 8C12F11

Order Number: U4466HJ110-1

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 8C12F11 were listed as below:

Heavy chain: DNA sequence (1389 bp)

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

ATGGAATGGACCTGGGTCTTTCTCTTCCTCCTGTCAGTAACTGCAGGTGTCCACTCC**CAGGTT**CAGC
TGCAGCAGTCTGGAGCTGAGCTGATGAAGCCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTACTGG
CTACACATT**CAGT**AGGT**ACT**GGATAGAGTGGGTAAAGCAGAGGCCTGGACATGGCCTTGAGTGGATA
GGAGAGATTTTACCTGGAAGTGGTAGAACTAATTACAATGAGAAGTTCAAGGGCAAGGCCACATTCA
CTGCAGATACATCCTCCAACACAGCCTACATGCAACTCAGCAGCCTGACATCTGAGGACTCTGCCGT
CTATTACTGTGCACCGGGACTGGGAGGGGGTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTC
ACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAA
CTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTG
GAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACT
CTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCC
ACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGTGGTTGTAAGCCTTGCA
ATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGCCCAAGGATGTGCTCACCATT
ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCA
GCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAG
CACTTTCCGCTCAGTCAGTGAAGTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAA
TGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGAC
CGAAGGCTCCACAGGTGTACACCATTCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCT
GACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGAATGGGCAGCCA
GCGGAGAACTACAAGAACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGC
TCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCT
GCACAACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGA

Heavy chain: Amino acid sequence (462 aa)

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

MEWTWVFLFLLSVTAGVHSQVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIG
EILPGSGRNTNYNEKFKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCAPGTGRGYAMDYWGQTSV

TVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYT
LSSSVTVPSSTWPSSETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI
TLTPKVTCVVVDISKDDPEVQFSWFVDDDEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFK
CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
AENYKNTQPIMDTDGSYFVYSKLNQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK-

Light chain: DNA sequence (717 bp)

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

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCCTGCTTCCAGCAGTGATGTTTTGA
TGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGAGATCAAGCCTCCATCTCTTGCAGATCTAG
TCAGAGCATTTTACATAGTAATGGAAACACCTATTTAGAAATGGTACCTGCAGAAACCAGGCCAGTCT
CCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTTCAAGTGGCAGTG
GATCAGGGACAGATTTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTG
CTTCAAGGTTACATGTTCTCGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTGAT
GCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
TGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACG
ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGC
ACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGA
CATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTAG

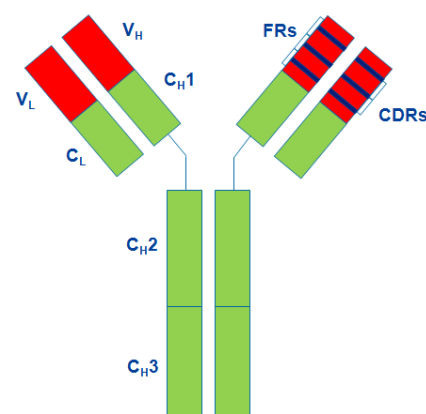
Light chain: Amino acid sequence (238 aa)

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

MKLPVRLLVLMFWIPASSSDVLMQTPLSLPVSLGDQASISCRSSQSILHSNGNTYLEWYLQKPGQS
PKLLIYKVSNRFSGVDPDRFSGSGSDFTLKISRVEAEDLGVYYCFQGSHVPRTFGGGTKLEIKRAD
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 IGHV1-9*01	productive	96.6% (281/291 nt)	Musmus IGHJ4*01	Musmus IGHD4-1*01, IGHD4-1*02	CAPGTG RGYAMD YW	in-frame
V _L	Musmus IGKV1-117*01	productive	99.7% (300/301 nt)	Musmus IGKJ1*01	-	CFQGSH VPRTF	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: 10/27/2022

Approved by

Bingying Luo

Luo Bingying

Antibody Department

GenScript USA Inc.