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Datasheet for 200-301-NJ3

Feimin Antibody [7F7.B2]

Overview

Description:	Anti-Feimin (MOUSE) Monoclonal Antibody - 200-301-NJ3
Item No.:	200-301-NJ3
Size:	100 µg
Applications:	ELISA, IP, SDS-PAGE, WB
Reactivity:	Human, Mouse
Host Species:	Mouse Balb/c

Product Details

Background:	The Feimin protein, also known as UPF0461 protein or C5orf24, involves regulatory roles within the cell and its exact role is under investigation. Feimin is a newly discovered myokine that complements insulin by fine-tuning glucose levels and boosting exercise capacity, especially under conditions where insulin is less effective. Highly conserved Feimin functions in both glucose homeostasis and has a direct role in muscle thermogenesis. Feimin can be secreted by muscle and act as a myokine where it can affect tissues by engaging MERTK receptor thereby activating AKT. Cellular Feimin (cFeimin) functions as a transcription regulator by interacting with FOXC2 protein, where it helps to enhance exercise performance by reducing thermogenesis.
Synonyms:	Chromosome 5 Open Reading Frame 24, UPF0461 Protein C5orf24, FLJ37562, Feimin
Host Species:	Mouse Balb/c
Clonality:	Monoclonal
Clone ID:	7F7.B2
Format:	IgG2b

Target Details

Gene Name:	C5orf24
Reactivity:	Human, Mouse
Immunogen Type:	Recombinant Protein

Immunogen:	This monoclonal antibody was prepared in mice after repeated immunizations with recombinant mouse Feimin protein.
Purity/Specificity:	Anti-Feimin Monoclonal Antibody is purified from tissue culture supernatant fluid by Protein A purified. A BLAST analysis of the sequence of the immunogen shows 100% identity with mouse, 97% bovine, and 94% human. This product reacts with mouse, and human Feimin isoform 1 and 2. Reactivity against homologues from other sources is not known.
Relevant Links:	• NCBI - NP_001129058.1 • GeneID - 134553 • UniProtKB - Q7Z6I8-1 (human) • UniProtKB - Q80X32 (mouse)

Application Details

Tested Applications:	ELISA, IP, SDS-PAGE, WB
Application Note:	Anti-Feimin (MOUSE) Antibody has been tested by ELISA, Western Blot, and Immunoprecipitation. In western blot, the antibody detects both human and mouse feimin protein from overexpressing cell lysates (using HEK293T). A band approximately 17kDa for isoform 2 and 20kDa for isoform 1 corresponding to C5orf24 is detected. Cell lines expressing feimin can be expected using appropriate tissues and lysates. In immunoprecipitation, the antibody detects mouse feimin. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:265,000-1:409,000
IP:	40ng/mL
WB:	1:5000

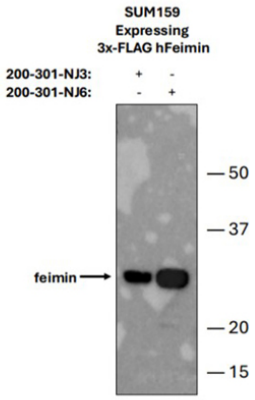
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.16 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

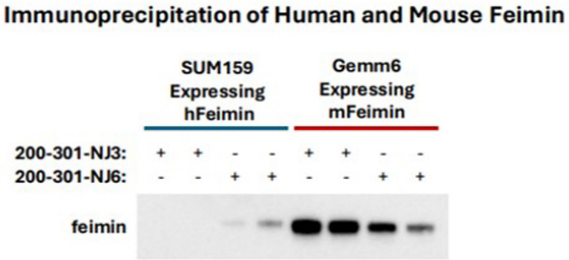
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store antibody at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. Antibody is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

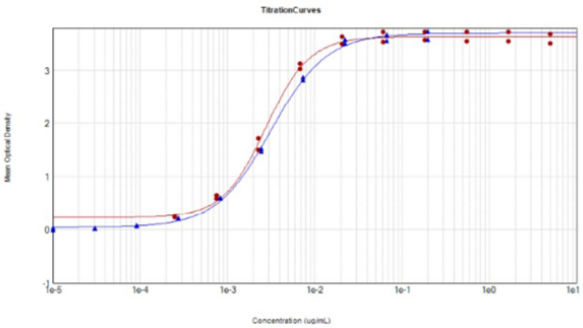
Images



Immunoprecipitation
Feimin Immunoprecipitation (IP).
Cell lysates (2x10^6 cells) from SUM156 cells overexpressing 3x FLAG-tagged human feimin were subjected to immunoprecipitation using 1µg/mL of mouse anti-feimin monoclonal antibodies (p/n 200-301-NJ3 and 200-301-NJ6). The immunoblot (PVDF) was then probed with 200ng/mL rabbit anti-feimin polyclonal antibodies (p/n 200-401-NH2). Both monoclonal antibodies successfully immunoprecipitated human feimin with 200-301-NJ6 showing greater recovery than 200-301-NJ3.
Image courtesy of Huachen Gan, Ph.D., Earl Labs, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill.

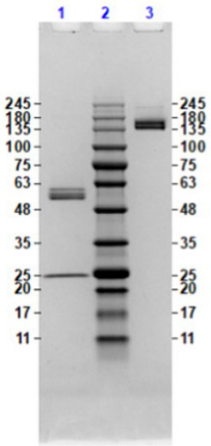


Immunoprecipitation
Immunoprecipitation of Human and Mouse Feimin.
Cell lysates (2x10^5 cells) from SUM159 cells overexpressing human feimin and Gemm6 cells overexpressing mouse feimin were subjected to immunoprecipitation using 1µg/mL of the mouse anti-feimin monoclonal antibodies (p/n 200-301-NJ3 and 200-301-NJ6). Immunoblots were then probed with 200 ng/mL of the rabbit anti-feimin polyclonal antibody (p/n 200-401-NH2). Monoclonal antibody 200-301-NJ6 immunoprecipitated human feimin, whereas both 200-301-NJ3 and 200-301-NJ6 immunoprecipitated mouse feimin.
Image courtesy of Huachen Gan, Ph.D., Earl Labs, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill.



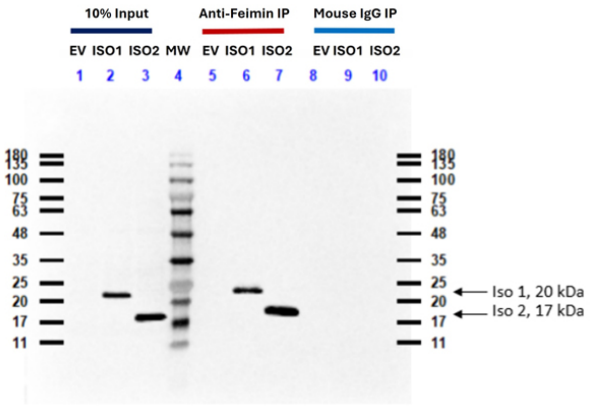
ELISA

ELISA results of Anti-Feimin (Mouse) Monoclonal Antibody Clone 7F7.B2. Each well was coated in duplicate with 10µg/mL of mouse Feimin. The starting dilution of antibody was 0.2µg/mL (blue) or 5µg/mL (red) and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. The antibody titer was 1:315,000. Assay performed using 3% Fish Gel in PBS (p/n MB-066), Rabbit Anti-Mouse IgG HRP conjugated (p/n 610-403-C46), and TMB substrate (p/n TMBE-1000).

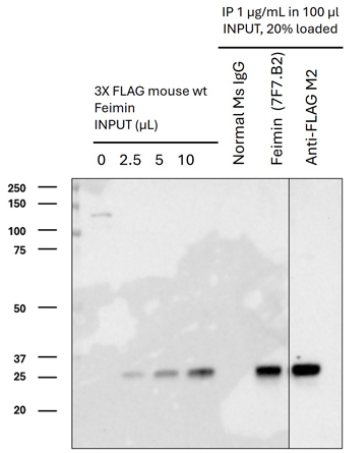


SDS-PAGE

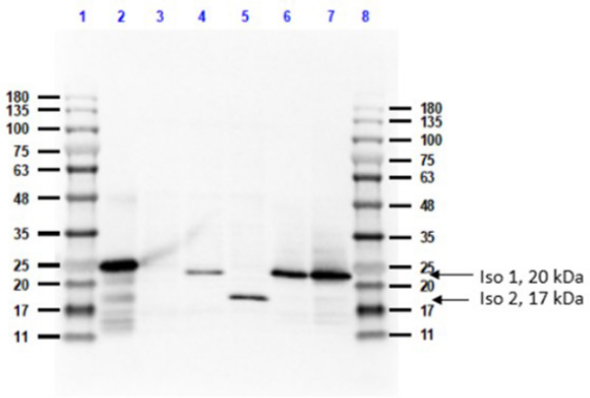
Coomassie stained SDS-PAGE results using Anti-Feimin (Mouse) Monoclonal Antibody (clone 7F7.B2) separated in a 4-20% gradient gel. Lane 1: Anti-Feimin (clone 7F7.B2) reduced [1.0µg]. Lane 2: Molecular weight standards. Lane 3: Anti-Feimin (clone 7F7.B2) non-reduced [1.0µg]. Results show purity with no signs of degradation or aggregation.



Immunoprecipitation
Feimin Immunoprecipitation (IP) followed by Western Blot (WB) analyses. Western Blot demonstrates Feimin at 10% input, Anti-Feimin-IP (p/n 200-301-NJ3), or mouse IgG-IP (p/n 010-0102) fractions in empty vector, isoform 1, or isoform 2 HEK293T transfected cells. Cells were lysed using cell lysis buffer (p/n MB-078-0050) and protein samples were incubated with anti-feimin antibody (200-301-NJ3) and protein-A beads (p/n PA50-00-0002) overnight at 4 °C, while gently rocking. The following day, beads were washed and proteins were dissociated from the beads and boiled prior to the Western Blotting analyses. Lanes 1-3: load 10% of input protein for immunoprecipitation. Primary Antibody: Anti-Feimin at 40ng/mL overnight at 2-8°C. Secondary Antibody: Anti-Rabbit TrueBlot HRP (p/n 18-8816-33) 1:40,000 at RT for 1 hr. Blocking Buffer: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) at RT for 30mins. Predicted MW: Isoform 1: ~20 kDa; Isoform 2: ~17 kDa.



Immunoprecipitation
Feimin Immunoprecipitation (IP) followed by Western Blot (WB) analyses. Western Blot demonstrates Feimin at 20% input, 3X FLAG mouse wt infected Gemm6 cell lysate, Feimin (clone 7F7.B2), mouse FLAG M2 antibody (positive control), or normal mouse IgG (negative control) fractions.
Primary antibody: polyclonal Anti-Feimin (p/n 200-401-NH2) at 200ng/mL overnight at 2-8°C.
Secondary Antibody: Anti-Rabbit IgG HRP at 1:10,000.
Predicted MW ~23kDa.
Image courtesy of Huachen Gan, Ph.D., Earl Labs, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill.



Western Blot
Western blot analysis of Anti-Feimin.
Lane 1 and 8: Molecular Weigh Markers.
Lane 2: Mouse Feimin N-term HIS, (+) [0.1µg].
Lane 3: HEK293T cells transfected, Empty Vector (-) [10µg].
Lane 4: HEK293T cells transfected, Hu feimin Isoform 1 (+) [10µg].
Lane 5: HEK293T cells transfected, Hu feimin Isoform 2 (+) [10µg].
Lane 6: HEK293T cells transfected, Ms feimin (+) [2µg].
Lane 7: HEK293T cells transfected, Ms feimin (+) [5µg].
Primary Antibody: Anti-Feimin at 1:5000 overnight at 2-8°C. Secondary Antibody: Rabbit Anti-Mouse HRP mx (p/n 610-403-C46) at 1:40,000 for 30 mins at RT. Blocking Buffer: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) for 30 mins at RT. Predicted MW: Expect 17kDa for Isoform 2, 20 kDa for Isoform 1.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.