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Product datasheet for AM20016AF-N

FHL2 Mouse Monoclonal Antibody [Clone ID: 11-134]

Product data:

Product Type:	Primary Antibodies
Clone Name:	11-134
Applications:	IHC, IP, WB
Recommended Dilution:	**Western blot:** 1-5 µg/ml *Positive Control:* C2C12 **Immunoprecipitation:** 3 µg/200-300 µl of cell extract. *Positive Control:* C2C12 **Immunohistochemistry:** 1-5 µg/ml Heat treatment is necessary for Paraffin Embedded Sections. Autoclave; 10 minutes in citrate buffer (pH 6.5) at 110°C *Positive Control:* Prostate cancer. Detailed procedure is provided in the following**Protocols.**
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	His-FHL2.
Specificity:	This antibody reacts with Mouse FHL2.
Formulation:	PBS, pH 7.2 containing 50% Glycerol without preservatives. State: Azide Free State: Liquid purified IgG fraction.
Concentration:	lot specific
Purification:	Protein-A Sepharose Chromatography.
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at -20°C.
Stability:	Shelf life: one year from despatch.
Gene Name:	four and a half LIM domains 2

Database Link: Entrez Gene 14200 MouseEntrez Gene 2274 Human Q14192

Background: Proteins containing LIM domains (which are double zinc finger motifs implicated in protein binding) are important regulators of cell growth, cell differentiation, and remodeling of the cell cytoskeleton. Human Four-and-a-half LIM-only protein 2 (FHL2), also known as DRAL/Slim3 is a 32 kDa protein expressed predominantly in human heart and to a lesser extent in skeletal muscle, testis, and prostate epithelium. Since FHL2 is abundant in heart tissue, it may play a role in the regulation of myofibrillogenesis of heart via LIM-domain binding to focal adhesions. FHL2 has also been identified as a co-activator of the androgen receptor where it promotes androgen receptor transcriptional activity. Stimulation of the Rho signaling pathway induces translocation of FHL2 to the nucleus and subsequent activation of FHL2- and androgen receptor-dependent genes. FHL2 also acts as a trancriptional repressor in muscle cells and is involved in modulation of beta-catenin-dependent transcription of Wnt-responsive genes.

Synonyms: FHL-2, SLIM 3, DRAL

Note: This product was originally produced by MBL International.

Protocol: **SDS-PAGE and Western Blotting**

1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make an 8 mg/mL solution.
3) Mix the sample with an equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for specific transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at RT or overnight at 4°C.
7) Incubate the membrane with the anti-FHL2 (11-134) monoclonal antibody (1-5 µg/mL) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10000 POD-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
10) Wash the membrane with PBS (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.

12) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.
Positive Controls for Western blotting: C2C12

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add 2 µg of the anti-FHL2 (11-134 monoclonal antibody into 250 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% Protein A-agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting.)**
Positive Controls for Immunoprecipitation: C2C12.

Immunohistochemical Staining for Paraffin-Embedded Sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
Heat treatment by Autoclave: Place the slides put on staining basket in 500 mLbeaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides with the autoclave for 10 min at 110°C. Let the slides cool down in the beaker at room temperature for about 40 min.
5) Remove the slides from the citrate buffer and cover each section with 3% H2O2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with the anti-FHL2 monoclonal antibody diluted with PBS containing 1% BSA (1-5 µg/mL).
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9.
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate

for 10 minutes at room temperature. Wash as in step 9.
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H2O2 in 150 mL PBS. *DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.
Positive Control for Immunohistochemistry: Prostate Cancer.

Protein Families: Druggable Genome

Product images:

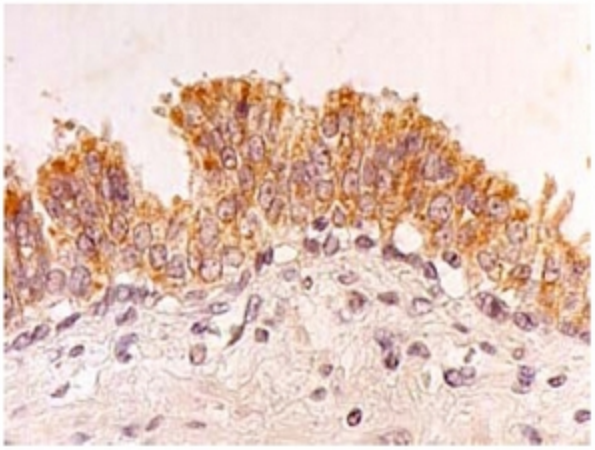

Figure 3. Immunohistochemical detection of FHL2 on Paraffin Embedded section of a Human prostate cancer with FHL2 antibody (AM20016AF-N).

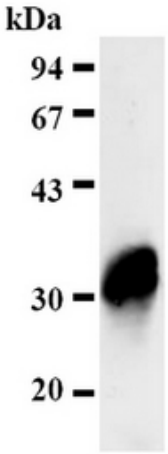

Figure 1. Western blot analysis of FHL2 expression in C2C12 using FHL2 antibody (AM20016AF-N).

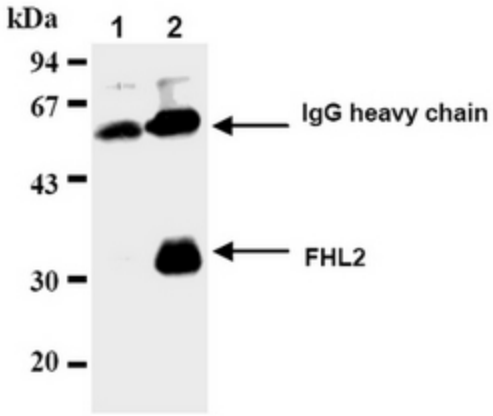

Figure 2. Immunoprecipitation of FHL2 from C2C12 cells with normal mouse IgG (Lane 1) or AM20016AF-N (Lane 2). After immunoprecipitated with the antibody, imminocomplex was resolved on SDS-PAGE and immunoblotted with FHL2 antibody (AM20016AF-N).