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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-FHL2 mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0055-3	11-134	Mouse IgG2a	100 µL	1 mg/mL

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 domains 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 coactivator 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 transcriptional repressor in muscle cells and is involved in modulation of β-catenin-dependent transcription of Wnt-responsive genes.

SOURCE: This antibody was purified from hybridoma (clone 11-134) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with the recombinant full-length human FHL2.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human and mouse FHL2 on Western blotting. This clone dose not cross-react with FHL1, FHL3, FHL4 and ACT.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell, Tissue	Prostate cancer	C2C12	Not tested
Reactivity	+ (IHC)	+ (WB)	

INTENDED USE:
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APPLICATIONS:
Western blotting; 1-5 µg/mL for chemiluminescence detection system
Immunoprecipitation; 3 µg/200-300 µL of cell extract
Immunohistochemistry; 1-5 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Autoclave; 10 minutes at 110°C in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1), 2) and 4).
Flow cytometry; Not tested
Chromatin immunoprecipitation
*It is reported that this antibody can be used in this application in the reference number 3), 7) and 9).

Detailed procedure is provided in the following **PROTOCOLS**.

REFERENCES:
1) Jin, H., *et al., Oncogene* (2016) In press. [WB, IC, IHC]
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3) Rachmin, I., *et al., Int. J. Cardiol.* **195**, 85-94 [WB, IP, ChIP]
4) Li, S. Y., *et al., J. Am. Soc. Nephrol.* **26**, 3072-3084 (2015) [WB, IC, IHC]
5) Hojayev, B., *et al., Mol Cell Biol.* **32**, 4025-4034 (2012) [WB, IHC]
6) Ewen, E. P., *et al., J. Biol. Chem.* **286**, 29644-29653 (2011) [WB, IHC]
7) Neuman, N. A., *et al., J. Biol. Chem.* **284**, 13202-13212 (2009) [ChIP]
8) Labalette, C., *et al., PLoS One* **3**, e3761 (2008) [WB]
9) Labalette, C., *et al., J. Biol. Chem.* **283**, 15201-15208 (2008) [ChIP]
10) Wang, J., *et al., Gastroenterology* **132**, 1066-1076 (2007) [WB, IHC]
11) Kahl, P., *et al., Cancer Res.* **66**, 11341- 11347 (2006)
12) Sun, J., *et al., Circ. Res.* **99**, 468-476 (2006) [WB, IP]
13) Weinert, S., *et al., J. Cell Biol.* **173**, 559-570 (2006) [WB]
14) Kang, D. E., *et al., J. Biol. Chem.* **280**, 31537-31547 (2005) [WB]
15) Müller, J. M., *et al., EMBO J.* **21**, 736-748 (2002)
16) Müller, J. M., *et al., EMBO J.* **19**, 359-369 (2000)

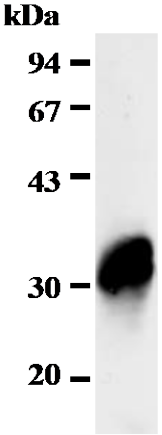
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume 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 cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 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/cm^2 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 precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 5 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; C2C12)



Western blot analysis of FHL2 expression in C2C12 cells using K0055-3.

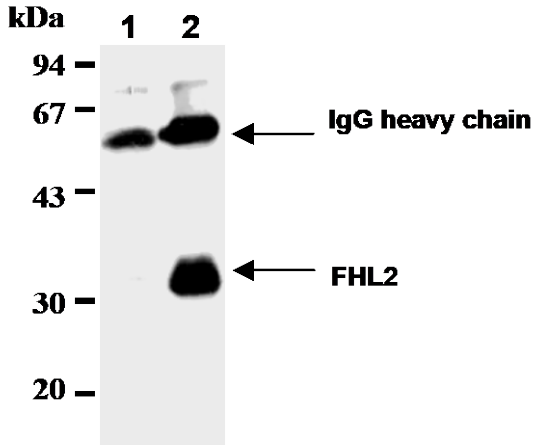
Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume 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 primary antibody as suggest in the **APPLICATIONS** into 200-300 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 cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the 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 control 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.



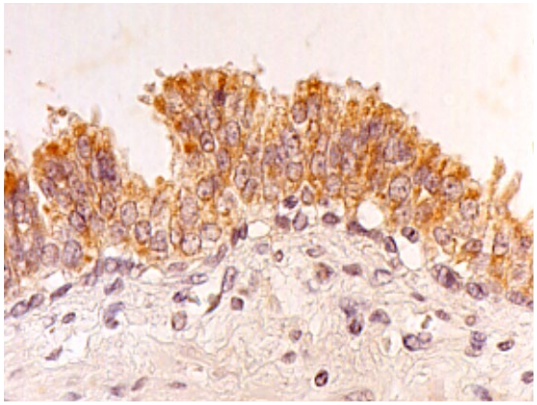
Immunoprecipitation of FHL2 from C2C12 cells with normal mouse IgG (1) or K0055-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with K0055-3.

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 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover

the beaker with plastic wrap, then process the slides with the autoclave for 10 minutes at 110°C. Let the slides cool down in the beaker at room temperature for about 40 minutes.

5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 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 (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
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 (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 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% H_2O_2 in 150 mL PBS. *DAB is a suspect 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)



Immunohistochemical detection of FHL2 on paraffin embedded section of a human prostate cancer with K0055-3.

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