V. 1.1

Anti-cofilin (rabbit origin)

Cat. # ACFL02

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

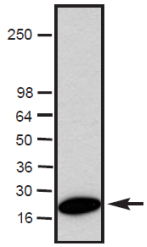
Material

ACFL02 is provided as an affinity purified rabbit polyclonal antibody. Cofilin is an actin binding protein that regulates actin dynamics and reorganization through monomer binding and severing of F-actin (reviewed in Bamburg 1999. Annu Rev Cell Dev Biol. 14:185-230). The antibody was raised against the N-terminal 13-22 amino acids of human cofilin1. Native cofilin has a predicted molecular weight of 19 kDa and migrates on an SDS-PAGE gel at 21 kDa (Yonezawa et al., 1987; Cell Structure and Function 12:443-452). Platelet cell extract (1 mg; Cat. # EXT01) is included as a Western blot positive control (see Fig. 1). ACFL02 is supplied as a lyophilized white powder.

Storage and Handling

Upon receipt, ACFL02 should be briefly centrifuged to collect the white powder at the bottom of the tube and stored desiccated at 4ºC. Reconstitute the antibody to 250 µg/ml by resuspending in 200 µl of PBS and store at 4ºC for up to one month. For storage longer than one month the antibody should be aliquoted, snap frozen in liquid nitrogen, and stored at -70ºC. Resuspend the platelet extract positive control protein in 500 µl of 1X SDS-PAGE sample buffer for a final concentration of 2 mg/ml, aliquot into 50 X 10 µl amounts (20 µg each), freeze in liquid nitrogen, and store at -70ºC. There is sufficient extract for 50 positive controls.

Figure 1. Western blot analysis of anti-cofilin antibody (Cat. #



ACFL02). Protein samples were separated by electrophoresis and transferred to PVDF membrane as described in the methods. ACFL02 antibody was diluted to 500 ng/ml (1:500) for Western blot analysis. Cofilin was detected in 20 µg of platelet cell extract (see arrow). Molecular weight markers are from Invitrogen.

Methods

Western blot analysis

Reagents:

1. Anti-cofilin antibody (Cat. # ACFL02)
2. SDS-PAGE and Western blot equipment
3. PVDF or Nitrocellulose membrane (Millipore Inc.)
4. Transfer Buffer (25 mM Tris-HCl, pH 8.3; 192 mM glycine, 5% methanol)
5. TBST: 10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20
6. Blotto: 5% non-fat dry milk in TBST
7. HRP goat anti-rabbit antibody (Jackson Labs)
8. Chemiluminescence detection reagents (Pierce SuperSignal West Dura Cat. # 34075)

Method:

1. Separate protein samples on a 4-20% SDS PAGE gel until the dye-front reaches the bottom of the gel.
2. Electroblot the proteins onto PVDF or Nitrocellulose membrane for 60 min at 350 mA with fresh Transfer Buffer.
3. Block the membrane in Blotto for 30 min at room temperature. (All remaining steps are at room temperature).
4. Probe with 500 ng/ml (1:500 dilution) of ACFL02 antibody in Blotto for 1 h. A 1:250 dilution of antibody can be used if protein samples have a low abundance of cofilin.
5. Wash the membrane three times with TBST for 5 min each.
6. Probe with 1:20,000 HRP anti-rabbit in Blotto for 1 h.
7. Wash the membrane six times with TBST for 5 min each.
8. Process the blots for chemiluminescence detection.
9. Typical assay results are shown in Figures 1 and 2.

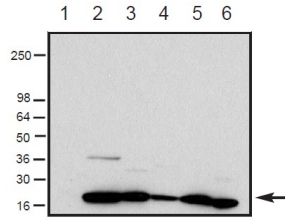


Figure 2. Western blot of cell extracts probed with anti-cofilin antibody (Cat. # ACFL02). Chemiluminescence detection of cofilin (see arrow) in 50 µg human HeLa cell extract (lane 5). Other lanes shown on the blot are Drosophila S2 (lane 1), Xenopus A6 (lane 2), mouse Swiss 3T3 (lane 3), rat NRK (lane 4), and human platelet (lane 6) cell extracts (50 µg each). Molecular weight markers are from Invitrogen.

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

Immunocytochemistry

Reagents:

1. Anti-cofilin antibody (Cat. # ACFL02)
2. Rhodamine conjugated anti-rabbit antibody
3. Wash Buffer (50 mM Tris pH 7.5, 150 mM NaCl)
4. Fixative Buffer (3.7% Formaldehyde in Wash Buffer, optional; add 0.1% glutaraldehyde) (see also Cat. # BK005)
5. Permeabilization Buffer (1% Triton X-100 in Wash Buffer; optional: use -20°C methanol instead but this is not compatible with rhodamine phalloidin staining)
6. Blocking Buffer (3% BSA in Wash Buffer)
7. 100 nM DAPI (4' 6-diamidino-2-phenylindole) in Wash Buffer
8. Polyvinyl alcohol antifade mounting medium with DABCO (Fluka Cat.# 10981)
9. Glass microscope slide (25 x 75 x 1 mm)

Method:

1. Grow tissue culture cells on glass coverslips until semi-confluent.
2. Remove culture media and gently wash the cells once with Wash Buffer.
3. Fix the cells with Fixative Buffer for 10 min.
4. Wash the cells three times with Wash Buffer.
5. Place the coverslips with the cell side up on parafilm inside of a petri dish. Maintain a humid atmosphere by placing a piece of wet filter paper inside the covered petri dish. Add 100 µl of Permeabilization Buffer to each coverslip and incubate for 20 min.
6. Remove Permeabilization Buffer, add 100 µl Block Buffer, and incubate for 30 min.
7. Wash the coverslips once with Wash Buffer.
8. Add 200 µl of 500 ng/ml (1:500 dilution) of ACFL02 antibody in Blocking Buffer to each coverslip. Incubate for 1 h.
9. Wash each coverslip three times in Wash Buffer (let stand for 5 min each).
10. Add 200 µl of a 1:500 dilution of rhodamine conjugated anti-rabbit antibody in Blocking Buffer to each coverslip. Incubate for 30 min.
11. Wash each coverslip three times in Wash Buffer (let stand for 5 min each).
12. Optional: Counterstain the DNA for 5 min with 200 µl of 100 nM DAPI in Wash Buffer.
13. Invert the coverslips on a drop of antifade mounting media on a glass slide. Gently remove the excess media with a tissue and allow mounting media to dry.
14. Store the slides in the dark at 4ºC.
15. Typical results are shown in Figure 3 with cofilin localized preferentially to cell protrusions and ruffles. These results are similar to previously published data (Yonezawa et al. 1987; Cell Structure and Function 12:443-452).

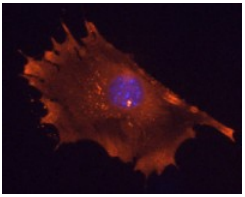


Figure 3. Immunofluorescence images of mouse Swiss 3T3 cells stained with anti-cofilin antibody (Cat. # ACFL02). Mouse Swiss 3T3 cells were grown to semi-confluency and fixed with 3.7% formaldehyde. Immunofluorescence staining using 500 ng/ml (1:500 dilution) ACFL02 antibody is shown (red). The primary antibody was detected with a 1:500 dilution of goat anti-rabbit rhodamine conjugated antibody. DNA (blue) was stained with 100 nM DAPI in PBS. Photograph was taken with a 20X objective lens.

Product Uses

This antibody is recommended for detection of cofilin in human, mouse, rat, and Xenopus (Fig. 2).

The following protocols have been tested with this antibody:

- Western blot analysis: recommended
- Immunoprecipitation: not recommended
- Immunocytochemistry: recommended for human, rodent, and Xenopus cells

Product Citations/Related Products

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