V. 1.0

Anti-Actin (rabbit origin)

Cat. # AAN01

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

Material

Rabbit polyclonal antibody against actin protein. Actin is the major protein of the microfilament cytoskeletal system and is a key protein in various cell motility processes. The immunogen used for antibody production was a peptide consisting of the 11 C-terminal amino acids of actin. Human platelet extract (Cat. # EXT01) is included as a positive control for Western blot analysis. A characteristic actin band at 43 kDa is identified on Western blots (see Fig. 1). Anti-actin antibody is supplied as a lyophilized white powder.

Storage and Reconstitution

Upon receipt, the product tube should be briefly centrifuged to collect the white powder at the bottom of the tube and stored desiccated at 4°C. Reconstitute the antibody to 500 µg/ml by resuspending in 200 µl of Milli-Q water plus 30% glycerol and store at 4°C for up to one month. To prevent bacterial growth at 4°C, add 50 µg/ml gentamicin sulfate or other antimicrobial agent. For storage longer than one month the antibody should be aliquoted, snap frozen in liquid nitrogen and stored at -70°C. Resuspend the Platelet Extract positive control protein in 500 µl of 1x SDS-PAGE sample loading buffer for a final concentration of 2 mg/ml, aliquot into 10 X 50 µl amounts (100 µg each) and store at -70°C.

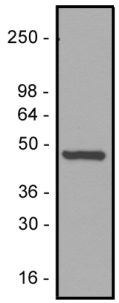

Figure 1. Western blot analysis of anti-actin antibody. Protein samples were separated by electrophoresis and transferred to PVDF membrane as described in the methods. Anti-actin antibody was diluted to 500 ng/ml (1:1000) for Western blot analysis. Actin was detected in 10 µg of platelet extract (43 kDa). Molecular weight markers are from Invitrogen.

Methods

Western blot analysis

Reagents:

1. Anti-actin antibody (Cat. # AAN01)
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-conjugated goat anti-rabbit antibody
8. Chemiluminescence detection reagents (ECL, Amersham Biosciences)

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.
4. Probe with 500 ng/ml (1:1000 dilution) of anti-actin antibody in TBST for 1 h. A 1:500 dilution of antibody can be used if protein samples have a low abundance of actin.
5. Wash the membrane three times with TBST for 5 min each.
6. Probe with 1:40,000 dilution of the anti-rabbit-HRP antibody in TBST for 1 h. A 1:20,000 dilution can used if protein samples have a low abundance of actin.
7. Wash the membrane six times with TBST for 5 min each.
8. Process the blots for chemiluminescence detection.
9. Typical results are shown in Figures 1 and 2.

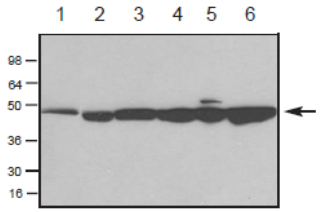

Figure 2. Western blot of purified actin and cell extracts probed with anti-actin antibody. Chemiluminescence detection of skeletal muscle actin (100 ng, lane 1), and in cell extracts of Xenopus A6 cells (lane 2), mouse Swiss 3T3 cells (50 µg, lane 3), rat NRK cells (50 µg, lane 4), human HeLa cells (50 µg, lane 5), and platelet cells (50 µg, lane 6). The actin band is indicated at 43 kDa (see arrow). The blot was probed with a 500 ng/ml (1:1000) dilution of anti-actin antibody.

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Immunocytochemistry

Reagents:

1. Tissue culture cells grown on glass coverslips (No. 1 thickness)
2. Anti-actin antibody
3. Rhodamine conjugated anti-rabbit antibody
4. Phosphate Buffered Saline (PBS) pH 7.4
5. 3% paraformaldehyde in PBS at room temperature
6. Permeabilization Buffer (1% Triton X-100 in PBS)
7. Blocking Buffer (3% BSA in 50 mM Tris pH 7.5)
8. 100 nM DAPI (4',6-diamidino-2-phenylindole) in PBS
9. Polyvinyl alcohol antifade mounting medium with DABCO (Fluka Cat. # 10981)
10. Glass microscope slide (25 x 75 x 1 mm)

Method:

1. Grow tissue culture cells on glass coverslips until 50% confluent.
2. Remove culture media and gently wash the cells once with isotemp PBS (37°C).
3. Fix the cells with 3% paraformaldehyde for 20 min at room temperature.
4. Wash the cells three times with PBS.
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 Blocking Buffer, and incubate for 30 min.
7. Wash the coverslips once with PBS.
8. Add 200 µl of 2 µg/ml (1:500 dilution) of anti-actin antibody in Blocking Buffer to each coverslip. A 1:100 dilution of antibody can be used for darker staining. Incubate for 1 h.
9. Wash each coverslip three times in Permeabilization 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 PBS (let stand for 5 min each).
12. Counterstain the DNA for 5 min with 200 µl of 100 nM DAPI in PBS.
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. Examine the stained coverslips using a fluoresence microscope equipped with filter sets suitable for rhodamine and DAPI fluorophores.
15. Store the slides in the dark at 4°C.
16. Typical results of actin staining are shown in Figure 3.

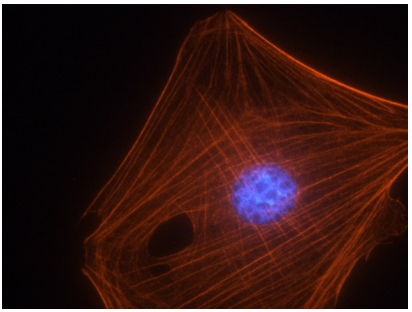

Figure 3. Immunofluorescence images of mouse Swiss 3T3 cells stained with anti-actin antibody. Swiss 3T3 cells were grown to 50% confluency and fixed with methanol. Immunofluorescence staining using 2 µg/ml (1:500 dilution) of anti-actin antibody is shown (red). The primary antibody was detected with 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 100X objective lens.

Product Uses

This antibody is recommended for detection of actin in human, mouse, rat, xenopus, and bovine extracts (Fig. 1 and 2).
The following protocols have been tested with this antibody:

- Western blot analysis: recommended
- Immunoprecipitation: not recommended

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.