V6.0

Anti-Pan Actin Mouse Monoclonal Antibody (Clone 7A8.2.1)

Cat. #: AAN02
Lot #:
Upon arrival, store at 4°C
See datasheet for storage after reconstitution

Form:	Lyophilized
Amount of material:	1 X 500 µl
Validated applications:	WB, IF
Species reactivity:	Broad cross species reactivity
Host/Isotype:	Mouse/IgG2b
Clone:	7A8.2.1

Background and Material Information

AAN02 is a mouse monoclonal 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 purified actin protein from rabbit skeletal muscle. A characteristic actin band at 43 kDa is identified on Western blots (Fig. 1). The antibody has been shown to recognize α–skeletal, α–cardiac, α–smooth muscle, β–cytoplasmic, γ–smooth muscle and γ– cytoplasmic actin isotypes (Fig. 1) and has broad species cross-reactivity. AAN02 is purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized antibody can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube.

Reconstitute each tube in 500 µl of 50% glycerol in deionized water (room temperature). We do not recommend using 50% glycerol at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Reconstituted antibody can be stored at 4° C for up to one month. For longer term storage the antibody should be aliquoted and stored at –20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 5% sucrose, and 1% dextran.

When stored and reconstituted as described, the product is stable for 6 months at -20°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock.

Applications

Western Blot (WB) Applications

Use as indicated below at 1:1000 dilution, sufficient for 500 ml of working strength Ab.

Western Blot Method:

1. Run protein samples and control samples in SDS-PAGE.
2. Equilibrate the gel in western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane for 60 min at 70 V.
4. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Block the membrane surface with 5% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
6. Incubate the membrane with a 1:1000 dilution of anti-actin antibody, diluted in 5% nonfat-dry milk in TBST, for 1 h at room temperature with constant agitation.
7. Rinse the membrane three times in 20-50 ml TBST for 10 min. each at room temperature with constant agitation.
8. Incubate the membrane with an appropriate dilution (e.g., 1:20,000 of anti-mouse secondary antibody (e.g., goat anti-mouse HRP conjugated IgG from Jackson Labs, Cat. # 115-035-068, in TBST/5% non-fat milk for 30 min shaking at room temperature.
9. Wash the membrane 5 times in TBST for 10 min each.
10. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate, Thermo Fisher).

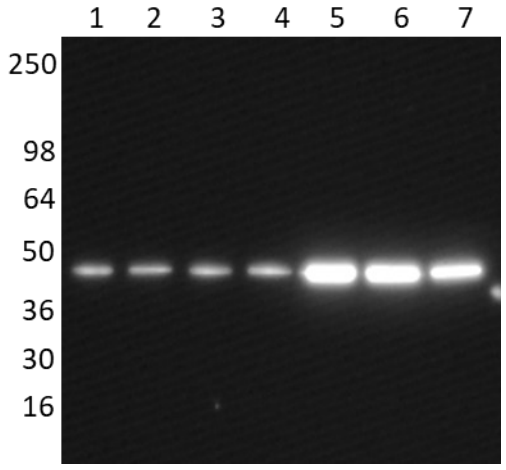

Figure 1. Western blot of purified actin and cell extracts probed with anti-actin antibody. Chemiluminescence detection of purified rabbit skeletal muscle actin (10 ng, Lane 1), purified bovine cardiac muscle actin (10 ng, Lane 2), purified chicken gizzard muscle actin (10 ng, Lane 3), purified non-muscle human platelet actin (10 ng, Lane 4), platelet cell extract (5 µg, Lane 5), platelet cell extract (5 µg, Lane 6), and A431 cell extract (5 µg, Lane 7). The actin band is indicated at 43 kDa. The blot was probed with a 1:1000 dilution of anti-actin antibody. 30 second exposure.

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Immunofluorescence (IF) Applications
Use as indicated below at 1:250-1:500 dilution, sufficient for 125-250 ml of working strength reagent.
NOTE: It is not recommended to fix cells in PFA for this Ab. Either methanol or a PFA/methanol treatment is recommended (Figures 2 & 3).

IF Method (PFA/Methanol/Triton X-100 Fixation of Swiss 3T3 cells):

1. 3T3 cells were plated on poly-lysine and laminin coated coverslips and grown to appropriate densities (~ 25% confluency).
2. Remove culture media and wash cells once with 1x PBS.
3. Fix cells for 10 min at room temperature (RT) with 3.7% PFA (paraformaldehyde in 1X PBS) (fresh, methanol free).
4. Wash cells two times with 1x PBS.
5. Permeabilize cells with 100% Methanol 4° C for 30 min.
6. Wash cells two times with 1x PBS.
7. Permeabilize cells with 0.5% Triton X-100 (in 1x PBS) for 15 min RT.
8. Wash cells two times with 1x PBS.
9. Block cells with 1% BSA, 0.3 M glycine in 1x PBS + 0.01% Tween (PBST) for 60 min RT.
10. Incubate cells with AAN02 (1: 250-1:500) in 1x PBS for 90 min at RT.
11. Wash cells once with 0.1% Triton-X-100 in 1x PBS.
12. Wash cells three times with PBST.
13. Incubate cells with (Alexa Fluor® 488) secondary antibody 1:500 in PBST for 60 min at RT (minimize light exposure).
14. Wash cells three times with 1x PBS.
15. Incubate cells with DAPI stain 1:500 in 1x PBS for 20 min at RT.
16. Wash cells three times with 1x PBS.
17. Dip coverslips in water.
18. Mount on glass slide with mounting media, and sit for 20 min.
19. Seal edges with nail polish for 10-20 min.

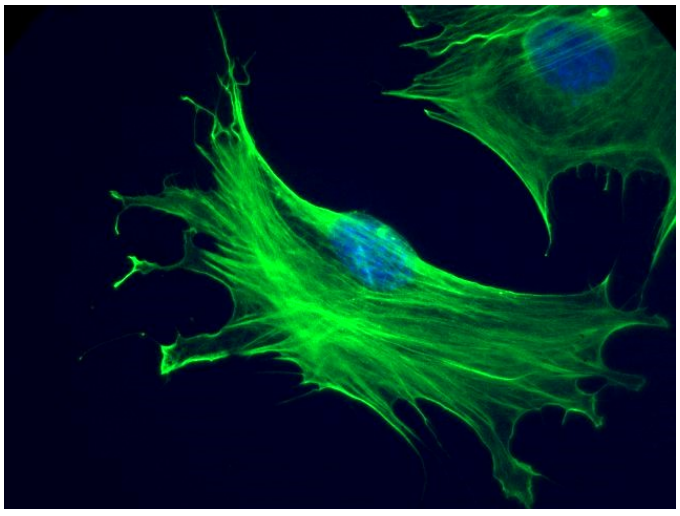

Figure 2. Immunofluorescence images of mouse Swiss 3T3 cells stained with anti-actin antibody. Swiss 3T3 cells were grown to 25% confluency on poly-lysine and laminin coverslips. 3T3 cells were fixed with PFA. Cells were permeabilized with methanol followed by 0.5% Triton X-100 as described in the method. Immunofluorescence staining using 1:500 dilution of anti-actin antibody in PBS is shown (green). The primary antibody was detected with a 1:500 dilution of goat anti-mouse Alexa Fluor® 488 conjugated antibody. DNA (blue) was stained with 50 nM DAPI in PBS. Image was taken with a 100X objective lens.

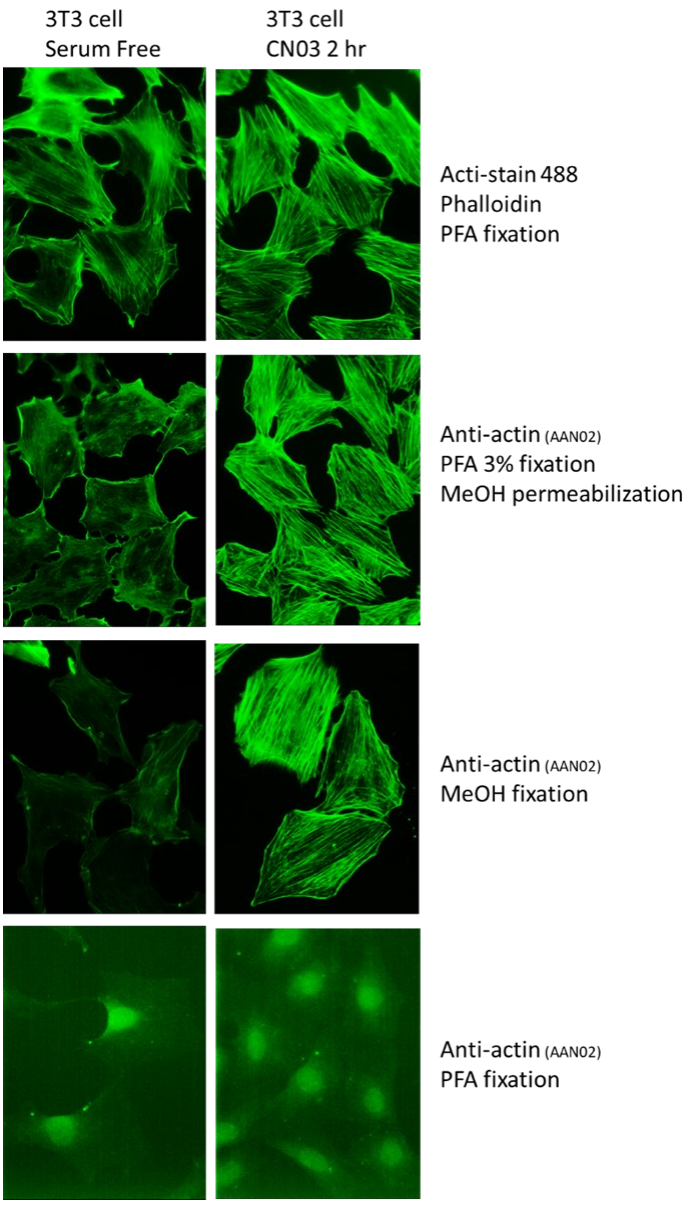

Figure 3. Comparison of fixation strategies used for Immunofluorescence images of mouse Swiss 3T3 cells stained with anti-actin antibody. Swiss 3T3 fibroblasts were plated on glass coverslips, grown to 30% confluency in DMEM plus 10% FBS, and serum starved for 24 h in media containing 1% FBS followed by 24 h in serum free media. Cells were treated with a buffer control or 1 µg/ml of the Rho Activator (CN03) for 2 h at 37°C/5% CO_2 to induce stress fibers. The 3T3 cells were then fixed with the following methods 1. PFA / phalloidin staining, 2. PFA-MeOH / AAN02 staining, 3. MeOH / AAN02 staining, 4. PFA / AAN02 staining. For condition 2, PFA fixation was followed by permeabilization with methanol as described in the method. Immunofluorescence staining using a 1:500 dilution of anti-actin antibody is shown (green). The primary antibody was detected with a 1:500 dilution of goat anti-mouse Alexa Fluor® 488 conjugated antibody. Images were taken with a 40X objective lens.

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