# Actin Phospho-Regulation

Immunocytochemistry Kit

**Cat. #** AK7710
**Size** Kit

| Cat. # | Description | Product Type | Size | Applications | Species Reactivity | ICC Dilution |
|---|---|---|---|---|---|---|
| AP1651 | Actin (N-terminal region) | Rabbit pAb | 50 µl | WB, ICC, E, IHC, IP | Hu, Rt, Ms, Ck | 1:50 |
| AP1671 | Actin (Tyr-53), phospho-specific | Rabbit pAb | 50 µl | WB, E, ICC | Hu, Rt, Ms, Ck | 1:50 |
| AM2021 | Actin (C-terminal region) | Mouse mAb | 50 µl | WB, E, ICC, IHC | Hu, Rt, Ms, Ck | 1:50 |
| MS3031 | Anti-Mouse Ig:DyLight® 594 | Goat pAb | 100 µl | ICC, IHC | Ms | 1:200 |
| RS3261 | Anti-Rabbit Ig:DyLight® 488 | Goat pAb | 100 µl | ICC, IHC | Rb | 1:200 |

Applications: WB = Western blot, E = ELISA, ICC = Immunocytochemistry, IP = Immunoprecipitation, IHC = Immunohistochemistry, FC = Flow Cytometry
Species: H = Human, R = Rat, M = Mouse, C = Chicken, F = Fish, Fr = Frog, Rb = Rabbit

## Kit Summary

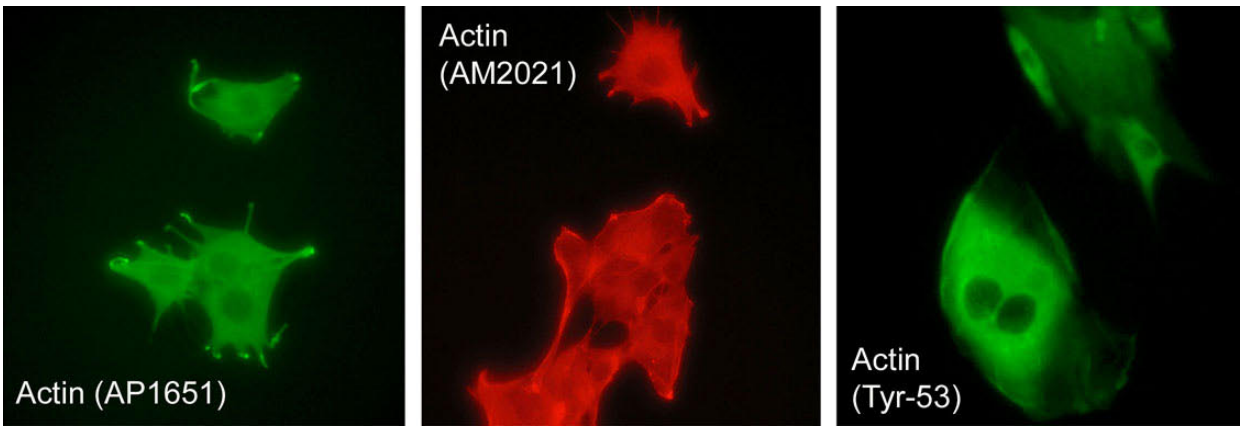
The actin phospho-regualtion kit can be used to label actin phosphorylation at Tyr-53 relative to total actin expression pattern. The kit contains anti-Actin (Tyr-53) phospho-specific antibody, and two antibodies for detecting total actin. In addition, secondary reagents conjugated to DyLight® 488 or DyLight® 594 are included for labeling primary antibodies for detection using green (493Ex/518Em) or red (593Ex/618Em) filter sets.

## Buffers and Storage

Mouse monoclonal, rabbit polyclonal, and secondary reagents are supplied in phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at –20°C. Stable for 1 year.

## Background

Actin is a major cytoskeletal protein involved in diverse cellular functions including cell motility, adhesion, and morphology. Six different actin isoforms have been identified in vertebrates. There are four α isoforms: skeletal, cardiac, and two smooth muscle (enteric and aortic) actins, along with two cytoplasmic actins (β and γ). Actin exists in two principal forms, globular monomeric (G) actin, and filamentous polymeric (F) actin. The assembly and disassembly of actin filaments, and also their organization into functional networks, is regulated by a variety of actin-binding proteins (ABPs). Phosphorylation may also be important for regulating actin assembly and interaction with ABPs. In Dictyostelium, phosphorylation of Tyr-53 occurs in response to cell stress and this phosphorylation may alter actin polymerization. In B cells, SHP-1 tyrosine dephosphorylation of actin leads to actin filament depolymerization following BCR stimulation.




Immunocytochemical labeling of phosphorylated actin in paraformaldehyde fixed and acetone permeabilized C2C12 cells treated with pervanadate. The cells were labeled with mouse monoclonal anti-Actin (AM2021) and rabbit polyclonal anti-Actin (AP1651) or anti-Actin (Tyr -53) (AP1671). The antibodies were detected using Goat anti-Mouse DyLight® 594 or Goat anti-Rabbit DyLight® 488.



www.ecmbio.com
info@ecmbio.com
toll-free: 1-800-859-8202
telephone: 859-879-2075

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Adherent Cell Fixation

1. Remove cell growth medium from culture plate containing cells, and rinse cells once with Hank's buffered saline solution (HBSS) or other rinse buffer acceptable for your cell type.

2. Fix cells with 4% Paraformaldehyde/0.2% NP-40 in HBSS for 30 minutes at room temperature.

   **Note:** Some antibodies work better for immunocytochemistry using one of the following methods:
   A. Methanol/Acetone fixation: Fix and permeabilize in 1:1 Methanol/Acetone at -20°C for 10 min.
   B. Aldehyde/Acetone fixation: Fix cells with 4% Paraformaldehyde in HBSS for 30 minutes at room temperature, then permeabilize for 15 min. with 100% Acetone for at -20°C.

3. Remove fixation solution and rinse cells two times with phosphate buffered saline solution (PBS).

4. Block non-specific binding sites with 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature.

   **Note**: Normal animal serum (e.g. horse, goat) that matches the species of the secondary antibody can be substituted for BSA, if non-specific labeling occurs with certain secondary reagents.

Primary Antibody Labeling

5. Make primary antibody dilutions in 1% BSA in PBS, using the recommended dilution described in the table above. For some cell types, the optimal antibody dilution may need to be empirically determined. Titrations of 1:50 to 1:500 can be useful to determine the optimal dilution for each primary antibody.

6. Remove the blocking solution from step #4, then add primary antibody dilutions and incubate for 1-2 hours at room temperature.

7. After primary antibody probing, rinse cells three times with PBS.

Secondary Antibody Labeling

8. Make secondary dilutions in 1% BSA (or normal serum) in PBS.

9. Suggested dilutions for secondary antibodies used at ECM Biosciences:

| | | | |
|---|---|---|---|
| RS3261 | Goat anti-Rabbit Ig:DyLight® 488 | (Green; Abs./Em. = 493/518) | 1:200 |
| MS3011 | Goat anti-Mouse Ig:DyLight® 488 | (Green; Abs./Em. = 493/518) | 1:200 |
| RS3271 | Goat anti-Rabbit Ig:DyLight® 594 | (Red; Abs./Em. = 593/618) | 1:200 |
| MS3031 | Goat anti-Mouse Ig:DyLight® 594 | (Red; Abs./Em. = 593/618) | 1:200 |

10. Add secondary antibody to cells for 30 minutes at room temperature.
    **Note**: Fluorescent secondary antibody labeling should be performed in the dark.

11. For long-term storage (months at 4°C), remove PBS and add SlowFade Gold (Invitrogen) to the cells and seal the slides or plates.





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