Cytoskeletal Filament Labeling

Immunocytochemistry Kit

Cat. # CK7720

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
PF7501	Phalloidin:FITC	Reagent	100 µl	ICC, IHC	Hu, Rt, Ms, Ck, F	1:500
TM4111	α-Tubulin (C-terminus)	Mouse mAb	50 µl	WB, E, IP, ICC, IHC	Hu, Rt, Ms	1:200
VM4341	Vimentin	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms, Ck	1:100
MS3031	Anti-Mouse Ig:DyLight® 594	Goat pAb	100 µl	ICC, IHC	Ms	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

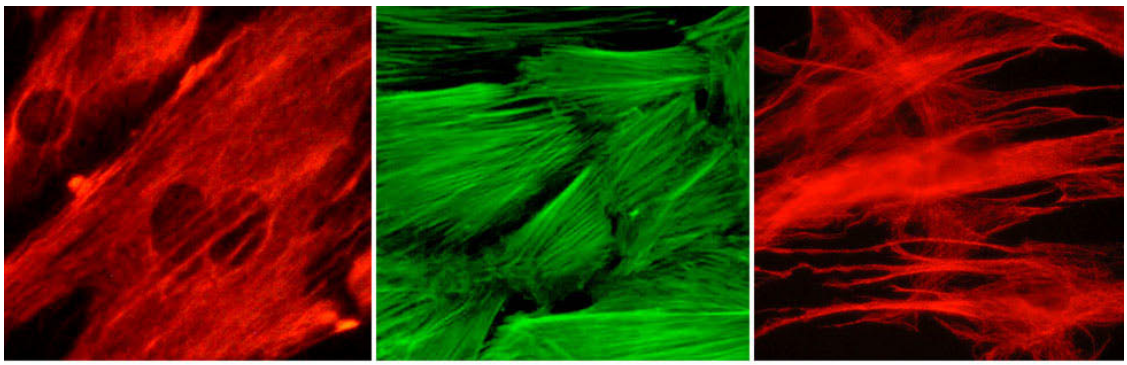
The cytoskeletal filament kit can be used to label the major cytoskeletal elements in cells: actin filaments (Phalloidin: FITC), intermediate filaments (Vimentin), and microtubules (α-tubulin). The kit includes Phalloidin:FITC and Goat anti-mouse conjugated to DyLight® 594 for for dual labeling experiments.

Buffers and Storage

Mouse monoclonal 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

The cytoskeleton includes actin filaments, microtubules, and intermediate filaments. These major cytoskeletal filaments are important for cell motility, cell adhesion, and cell morphology. 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). MTs are dynamic polymers of α/β-tubulin heterodimers. At least two populations of MTs, called dynamic and stable according to their rates of turnover, are readily distinguishable in cells. The proteins associated with MTs (MAPs) are among the best-known factors that regulate MT dynamics and stability. Intermediate filament (IF) proteins are a diverse family with distinct expression patterns and functions. One major IF protein is vimentin, which forms filaments in mesenchymal, endothelial, and hematopoietic cells.



Immunocytochemical labeling of vimentin (VM4341; left), Phalloidin:FITC (PF7501; middle), and α-Tubulin (TM4111; right). The antibodies were detected using Goat anti-Mouse conjugated to DyLight® 594.

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ECM Biosciences

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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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