Paxillin Phospho-Regulation

Immunocytochemistry Kit

Cat. # PK7660
Size Kit

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
PM1071	Paxillin	Mouse mAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms, Ck	1:100
PP1341	Paxillin (Ser-83), phospho-specific	Rabbit pAb	50 µl	WB, E, ICC	Rt, Ms	1:50
MS3011	Anti-Mouse Ig:DyLight® 488	Goat pAb	100 µl	ICC, IHC	Ms	1:200
RS3271	Anti-Rabbit Ig:DyLight® 594	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

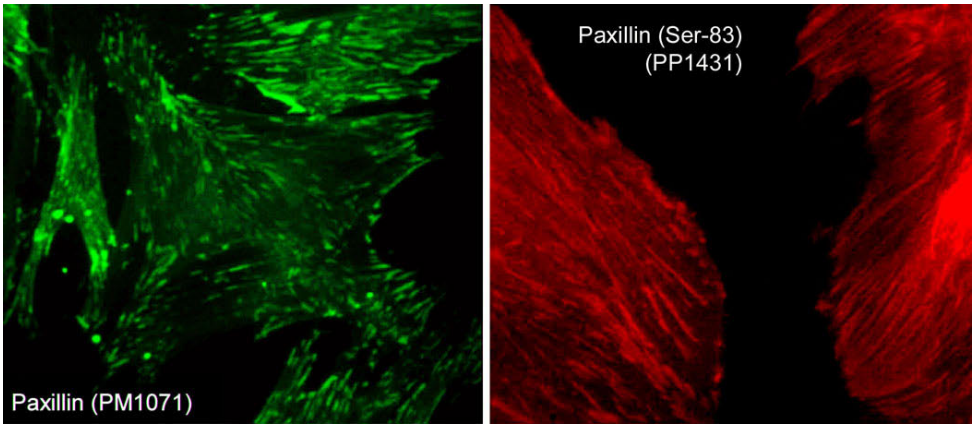
The paxillin phospho-regulation kit can be used for immunocytochemical co-localization of paxillin phosphorylation at Ser-83 compared to the total expression of paxillin. The kit includes rabbit polyclonal and mouse monoclonal antibodies along with Goat-anti-rabbit conjugated to DyLight® 594 and Goat anti-mouse conjugated to DyLight® 488 for dual labeling experiments.

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

Paxillin, a focal adhesion protein, is involved in focal adhesion formation during cell adhesion and migration. Paxillin contains LD motifs, LIM domains, and SH3-/SH2-binding domains that participate in a variety of protein-protein interactions with kinases, GTPase-activating proteins, and cytoskeletal proteins. Phosphorylation of paxillin occurs at both tyrosine and serine sites. Serine phosphorylation of paxillin occurs in response to growth-factor activation and fibronectins. Both ERK and p38MAPK kinases phosphorylate serine 83 in vitro. HGF stimulation of murine epithelial cells leads to ERK-mediated phosphorylation of Ser -83, which is required for HGF-induced cell spreading and migration. In addition, Ser-83 is phosphorylated in response to NGF in PC12 cells, and this phosphorylation may be involved in neurite extension. In human paxillin, Ser-85 rather than Ser-83 may be the site phosphorylated by p38 MAPK and mutation of this site inhibits NGF-induced neurite extension. Thus, serine residues in the N-terminal region of paxillin may be important for growth-factor mediated changes in activity.



Immunocytochemical labeling of phosphorylated paxillin in paraformaldehyde fixed and NP-40 permeabilized rat A7r5 cells. The cells were labeled with mouse monoclonal Paxillin (PM1071; left) and rabbit polyclonal Paxillin (Ser-83) (PP1341, right). The antibodies were detected with Goat anti-Mouse DyLight® 488 and Goat anti-Rabbit DyLight® 594, respectively.

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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