γ-Catenin Phospho-Regulation

Immunocytochemistry Kit

Cat. # CK7680
Size Kit

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
CM1111	γ-Catenin (C-terminal)	Mouse mAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms	1:50
CP1121	γ-Catenin (Tyr-550), phospho-specific	Rabbit pAb	50 µl	WB, E, ICC	Hu, 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 γ-Catenin phospho-regulation kit can be used for immunocytochemical co-localization of γ-Catenin phosphorylation at Tyr-550 compared to the total expression of γ-Catenin. 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.

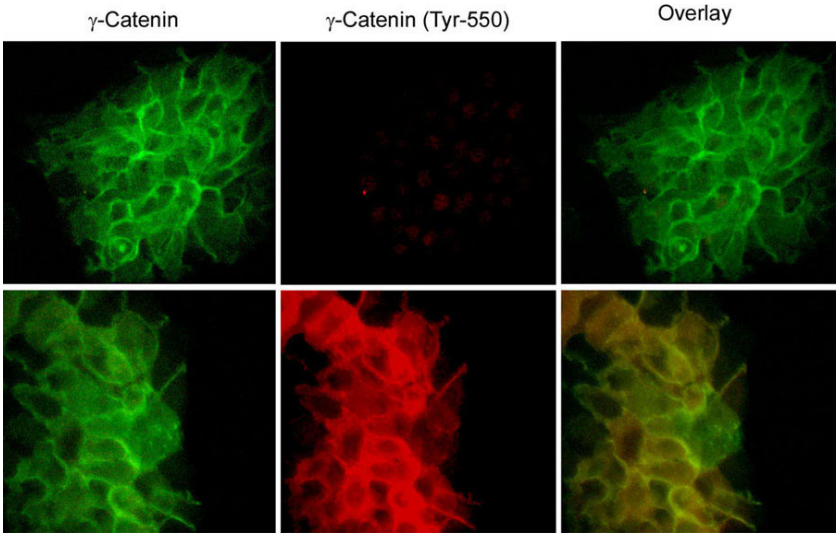
Buffers and Storage

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

Plakoglobin (γ-Catenin) is a catenin family member identified as a component of desmosomes. γ-Catenin has high homology to β-catenin and, like β-catenin, it can associate with the cadherins, E-cadherin and N-cadherin. One molecule of α-catenin and at least one molecule of β-catenin and γ-Catenin simultaneously bind to a single cadherin molecule. A 19-amino acid sequence of desmoglein was found to be critical for binding of γ-Catenin. Similar catenin-binding domains found in cadherins suggest a common mechanism for γ-Catenin localization to both adherens junctions and desmosomes. Phosphorylation of tyrosine residues in γ-Catenin can modify its interactions with other proteins. Phosphorylation of tyrosine 644 decreases γ-Catenin association with α-catenin, but increases binding to desmoplakin. Fer kinase can phosphorylate tyrosine 550, which increases γ-Catenin binding to α-catenin. Thus, tyrosine phosphorylation may be important for regulation of γ-Catenin protein-protein interactions within desmosomal complexes.

Immunocytochemical labeling of phosphorylated γ-Catenin in control (Top) and pervanadate-treated (Bottom) A431 cells. The cells were labeled with mouse monoclonal γ-Catenin (CM1111) and rabbit polyclonal γ-Catenin (Tyr -550) antibodies, then the antibodies were detected using Goat anti-Mouse DyLight® 488 and Goat anti-Rabbit DyLight® 594. The overlay showing co-labeling between the two antibodies is shown to the right.



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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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