δ1-Catenin Phospho-Regulation
Immunocytochemistry Kit

Cat. # CK7650

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
CM3541	δ1-Catenin (a.a. 275-285)	Mouse mAb	50 µl	WB, E, ICC, IP	Hu, Rt, Ms	1:100
CM3561	δ1-Catenin (Tyr-228), phospho-specific	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:100
CM3571	δ1-Catenin (Tyr-280), phospho-specific	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:100
CM3601	δ1-Catenin (Tyr-302), phospho-specific	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:100
MS3011	Anti-Mouse Ig:DyLight® 488	Goat pAb	100 µl	ICC, IHC	Ms	1:200
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

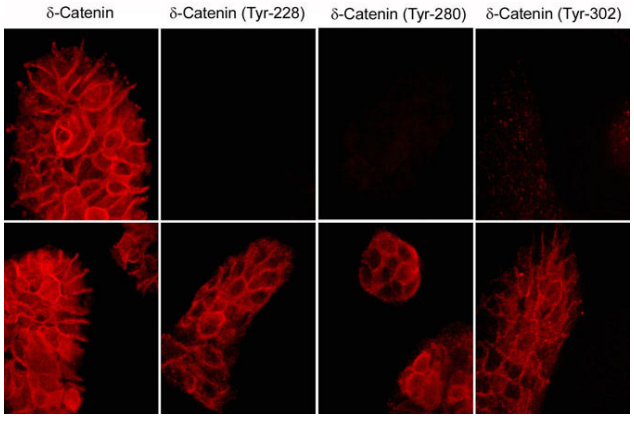
The δ1-Catenin phospho-regulation kit can be used for immunocytochemical labeling of phosphorylation at Tyr -228, Tyr-280, and Tyr-302 in δ1-Catenin. The kit includes a monoclonal antibody to examine the pattern of expression of the total population of δ1-Catenin. In addition, secondary reagents conjugated 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 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

Catenins have emerged as molecular sensors that integrate cell-cell junctions and cytoskeletal dynamics with signaling pathways that control morphogenesis and cell to cell communication. δ1-Catenin (p120 catenin) is a catenin family member which contains an N-terminal coiled-coil domain, a regulatory domain containing multiple phosphorylation sites, and a central Armadillo repeat domain. δ1-Catenin regulates E-cadherin turnover, and has both positive and negative effects on cadherin-mediated adhesion. Actin dynamics are also regulated by δ1-Catenin, which can modulate RhoA, Rac, and cdc42 activity. δ1-Catenin is phosphorylated at multiple tyrosine, serine, and threonine sites both in vitro and in vivo. High levels of δ1-Catenin phosphorylated at Tyr-228 are commonly seen in several carcinoma cell lines and after EGFR activation. Many other tyrosine sites are also phosphorylated in the N-terminal region, including Tyr-96, Tyr-112, Tyr-280, and Tyr-302. In addition, Thr-310 and Thr-916 are constituitively phosphorylated in many cell types, however this phosphorylation may occur only in δ1-Catenin associated with the plasma membrane.



Immunocytochemical labeling of phosphorylated δ1-Catenin in A431 control (top) or treated with pervanadate (bottom). The fixed cells were labeled with mouse monoclonal antibodies to δ1-Catenin (a.a. 275-285), δ1-Catenin (Tyr-228), δ1-Catenin (Tyr-280), and δ1-Catenin (Tyr-302). The antibodies were detected using Goat anti-Mouse secondary antibodies 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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