Phospho-Serine, -Threonine, & -Tyrosine
Immunocytochemistry Kit

Cat. # PK7740

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
PM3801	Anti-Phosphoserine/threonine	Mouse mAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms	1:50
PP2551	Anti-Phosphoserine/threonine	Rabbit pAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms	1:50
PM3751	Anti-Phosphotyrosine	Mouse mAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms, Ck	1:100
PP2221	Anti-Phosphotyrosine	Rabbit pAb	50 µl	WB, E, IP, ICC	Hu, Rt, Ms, Ck	1:100
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

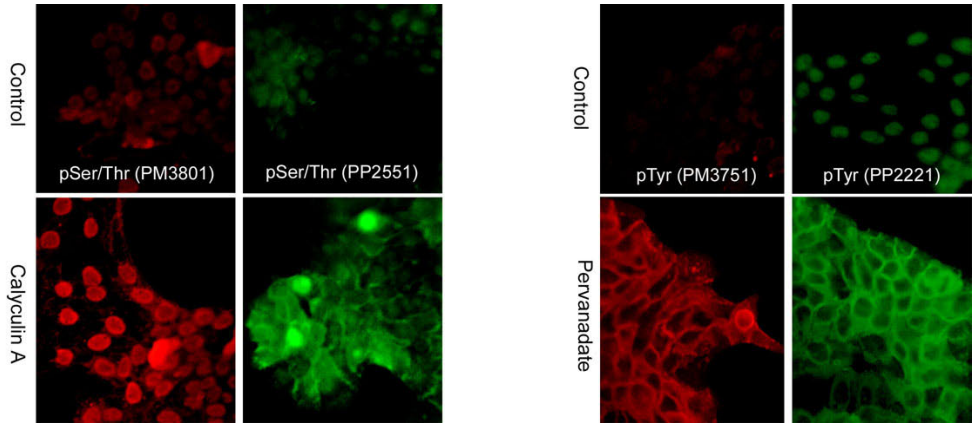
The phospho-serine, -threonine, and -tyrosine kit can be used to examine the pattern of phosphoserine-threonine relative to phosphotyrosine, or relative to a target protein. The kit includes monoclonal and polyclonal antibodies to phosphoserine-threonine and phosphotyrosine along with Goat-anti-Rabbit conjugated to DyLight® 488 and Goat anti-Mouse conjugated to DyLight® 594 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

Phosphorylation of specific tyrosine, serine, and threonine residues is an important post-translational modification for regulating the activity of proteins. Stimulation of a variety of cell signaling pathways activates the receptor and non-receptor kinases that mediate these protein modifications. Antibodies that can detect phosphotyrosine, phosphoserine, or phosphothreonine residues are excellent tools for characterizing changes in the post-translational state of a broad range of phospho-proteins. Immunoprecipitation of proteins of interest, followed by detection of phosphorylation using anti-phosphotyrosine or anti-phosphoserine/threonine antibody is commonly used to correlate changes in phosphorylation state with alterations in protein activity. Immunocytochemistry can also be used to examine changes in the level and localization of phosphotyrosine, phosphoserine, or phosphothreonine in cells after drug stimulation or during specific cell states.



Immunocytochemical labeling of phosphorylation in A431 treated with calyculin A (left panel) or pervanadate (right panel). The fixed cells were labeled with anti-Phosphoserine/threonine (PM3801) and anti-Phosphoserine/threonine (PP2551) (left panel) or with anti-Phosphotyrosine (PM3751) and anti-Phosphotyrosine (PP2221) (right panel). The antibodies were detected using appropriate secondary antibody conjugated to DyLight® 488 or 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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