ERK1/2 Phospho-Regulation

Immunocytochemistry Kit

Cat. # EK7620
Size Kit

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
EM2331	ERK1 (C-terminal region)	Mouse mAb	50 µl	WB, E, ICC, IHC	Hu, Rt, Ms	1:100
EM2061	ERK1 (Thr-202/Tyr-204)[conserved], 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

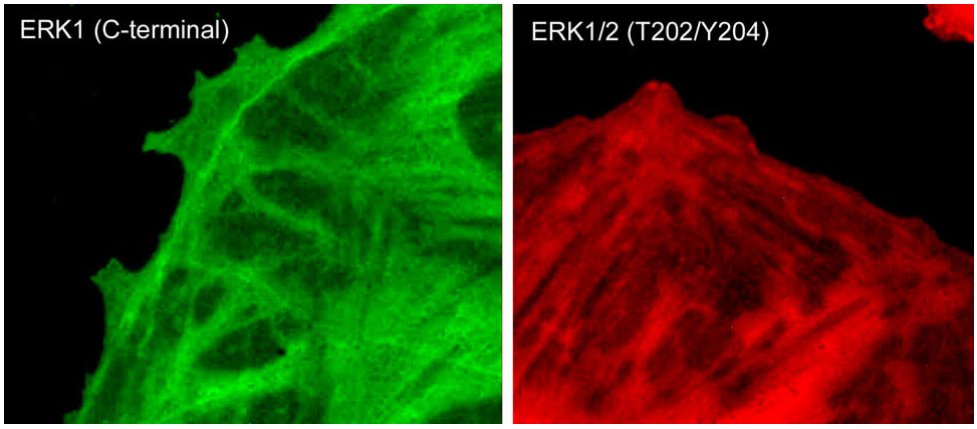
The ERK1/2 phospho-regulation kit may be used to examine the pattern of phosphorylation of ERK1 at the dual site (Thr-202/Tyr-204) relative to the total ERK1 pattern. The kit includes monoclonal antibodies to ERK1 (C-terminal region) and ERK1/2 (Thr-202/Tyr-204), as well as Goat anti-mouse secondary reagents conjugated to DyLight® 488 or DyLight® 594 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

Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The ERK1/2 (p44/42) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines. Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase (MAPK). Multiple ERK1/2 MAPKKKs have been identified, including members of the Raf family as well as Mos and Tpl2/Cot. MEK1 and MEK2 are the primary MAPKKs in this pathway. MEK1 and MEK2 activate ERK1 and ERK2 through phosphorylation of activation loop residues Thr-202/Tyr-204 and Thr-185/Tyr-187, respectively. ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases. Several downstream targets of ERK1/2 have been identified, including p90RSK and the transcription factor Elk-1.



Immunocytochemical labeling of phosphorylated ERK1 in paraformaldehyde-fixed and NP-40-permeabilized rat A7r5 cells treated with calyculin A. The fixed cells were labeled with mouse monoclonal antibodies to anti-ERK1 (EM2331) and anti-ERK1/2 (Thr-202/Tyr-204) (EM2061). The antibodies were detected using Goat anti-Mouse secondary antibodies conjugated to DyLight® 488 (left) and DyLight® 594 (right).

www.ecmbio.com
info@ecmbio.com
toll-free: 1-800-859-8202
telephone: 859-879-2075

Rev 4/15/2019

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.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.