Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antibody

Monoclonal Rabbit IgG Clone # 269434
Catalog Number: MAB1018

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat ERK1 and ERK2 dually phosphorylated at T202/Y204 or T185/Y187, respectively.
Source	Monoclonal Rabbit IgG Clone # 269434
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing ERK1 T202/Y204 site
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

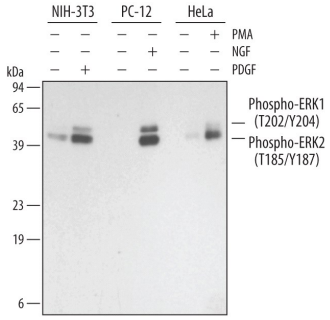
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	5 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

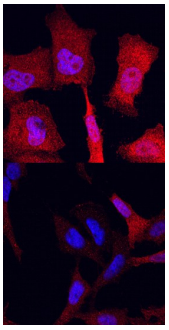
DATA

Western Blot



Detection of Mouse, Rat, and Human Phospho-ERK1 (T202/Y204) and ERK2 (T185/Y187) by Western Blot. Western blot shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 100 ng/mL Rabbit Anti-Human PDGF (Catalog # 120-HD) and PC-12 rat adrenal pheochromocytoma cell line untreated or treated with 100 ng/mL Recombinant Rat β-NGF (Catalog # 556-NG) and HeLa human cervical epithelial carcinoma cell line untreated or treated with 200 nM PMA. PVDF membrane was probed with 0.5 µg/mL of Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Monoclonal Antibody (MAB1018), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for Phospho-ERK1 (T202/Y204)/ ERK2 (T185/Y187) at approximately 40-45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Immunocytochemistry



Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) in HeLa Human Cell Line. ERK1/ERK2 phosphorylated at T202/Y204 (ERK1) and T185/Y187 (ERK2) was detected in immersion fixed Hela human cervical epithelial carcinoma cells, unstimulated (lower panel) and stimulated (upper panel) with PMA using Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Monoclonal Antibody (Catalog # MAB1018) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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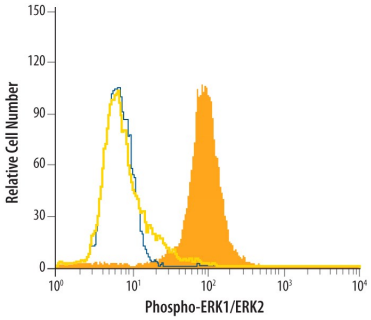
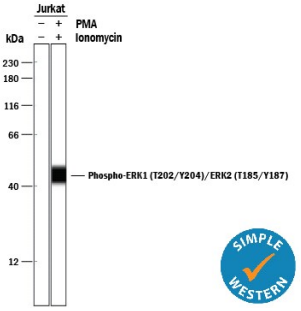
Intracellular Staining by Flow Cytometry



Detection of Phospho-ERK1/ERK2 in PMA-treated Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line were unstimulated (light orange open histogram) or treated with 50 ug/mL PMA for 10 minutes (dark orange filled histogram), then stained with Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Monoclonal Antibody (Catalog # MAB1018) or control antibody (Catalog # AB-105-C, blue open histogram), followed by Fluorescein-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0112). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with ice-cold methanol.

Simple Western



Detection of Human Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 200 nM PMA and Ionomycin for 20 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) at approximately 42 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Monoclonal Antibody (Catalog # MAB1018). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

ERK1 and ERK2 (also known as MAPK3 and MAPK1) are 44- and 42-kDa Ser/Thr kinases, respectively. They are part of the Ras-Raf-ERK signal transduction cascade often found downstream of growth factor receptor activation. ERK1 and ERK2 were initially isolated and cloned as kinases activated in response to insulin and NGF. They are expressed in most, if not all, mammalian tissues. Dual threonine and tyrosine phosphorylation activate both ERKs, at Thr202/Tyr204 for human ERK1 and Thr185/Tyr187 for human ERK2. The two proteins share 83% amino acid identity, differing mainly at the N and C termini.

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