

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat Phospho-ERK1/ERK2 when dually phosphorylated at T202/Y204 and T185/Y187, respectively.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing ERK1 T202/Y204 sites
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 either lyophilized or 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	0.1 µg/mL	See Below
Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
Immunohistochemistry	5-15 µg/mL	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 Human Phospho-ERK1 (T202/Y204) and ERK2 (T185/Y187) by Western Blot.
Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 200 nM PMA for the indicated times. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for Phospho-ERK1 (T202/Y204) and ERK2 (T185/Y187) at approximately 42 and 44 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry

Detection of Phospho-ERK1/ERK2 in Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line were untreated (yellow line open histogram) or treated with 200 ng/mL PMA for 15 minutes (filled histogram) and stained with Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018) or control antibody (Catalog # AB-105-C, blue line open histogram), followed by Fluorescein-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0112). To facilitate intracellular staining, cells were fixed with PFA and permeabilized with ice-cold methanol.

Immunohistochemistry

ERK1/ERK2 in Rat Brain. ERK1/ERK2 was detected in perfusion fixed frozen sections of rat brain (cortex) using 15 µg/mL Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018) overnight at 4 °C. Tissue was stained with the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Simple Western

Detection of Human Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with PMA, loaded at 0.2 mg/mL. A specific band was detected for ERK1 (T202/Y204)/ERK2 (T185/Y187) at approximately 44 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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. <ul style="list-style-type: none">● 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 is a protein Serine/Threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK1 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK1 is ubiquitously distributed in tissues with the highest expression in heart, brain, and spinal cord. Activated ERK1 translocates into the nucleus where it phosphorylates various transcription factors.

References:

1. Roskoski Jr., R. (2012) Pharmacol Res. **66**:105.