

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat MKK4 when dually phosphorylated at sites corresponding to S257 and T261 of human MKK4.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human MKK4 S257/T261 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 either lyophilized or as a 0.2 µm filtered solution in PBS.

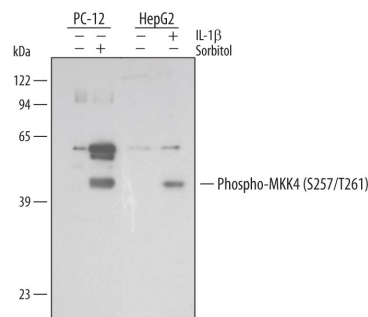
APPLICATIONS

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	1 µg/mL	See Below
Immunocytochemistry	3-15 µg/mL	Immersion fixed HeLa Human Cervical Epithelial Carcinoma Cells (Positive control) and HeLa Human Cervical Epithelial Carcinoma Cells treated with CIP (Calf Intestinal Phosphatase) (Negative Control)

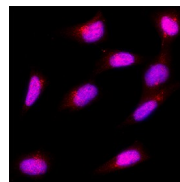
DATA

Western Blot

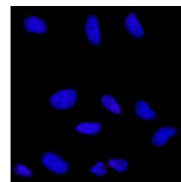


Detection of Rat and Human Phospho-MKK4 (S257/T261) by Western Blot. Western blot shows lysates of PC-12 rat adrenal pheochromocytoma cell line untreated (-) or treated (+) with 300 mM sorbitol for 30 minutes and HepG2 human hepatocellular carcinoma cell line untreated or treated with 10 ng/mL Recombinant Human IL-1β (Catalog # 201-LB) for 20 minutes. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-MKK4 (S257/T261) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2990), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-MKK4 (S257/T261) at approximately 44 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



Positive (HeLa cells)



Negative (HeLa+CIP cells)

Detection of MKK4 in HeLa Cells (Positive Control) and HeLa Cells treated with CIP (Calf Intestinal Phosphatase) (Negative Control). MKK4 was detected in immersion fixed HeLa Human Cervical Epithelial Carcinoma Cells (Positive control) and absent in HeLa Human Cervical Epithelial Carcinoma Cells treated with CIP (Calf Intestinal Phosphatase) (Negative Control) using Rabbit Anti-Human/Mouse/Rat Phospho-MKK4 (S257/T261) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2990) at 3 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 nuclei and cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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

Mitogen-activated protein kinase kinase 4 (MKK4 or MAP2K4), also known as SAPK/ERK kinase 1 (SEK1) and Jun kinase kinase 1 (JNKK1), is activated by proinflammatory cytokines and environmental stresses. Activation occurs through phosphorylation at Ser257 and Thr261 by several upstream MAPK kinase kinases (MAP3Ks). MKK4 is a dual specificity protein kinase, phosphorylating and activating the JNK family of MAP kinases at Thr and Tyr positions within the phosphoacceptor sequence Thr-Pro-Tyr.

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