Human/Mouse/Rat Phospho-JNK (T183/Y185) Antibody

Recombinant Monoclonal Rabbit IgG Clone # 1006A
Catalog Number: MAB1205

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat p46 and p54 JNK when dually phosphorylated at sites homologous to T183/Y185 of JNK1 and JNK2, and T221/Y223 of JNK3 in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1006A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Phosphopeptide containing human, rat, and mouse JNK1 T183/Y185 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.

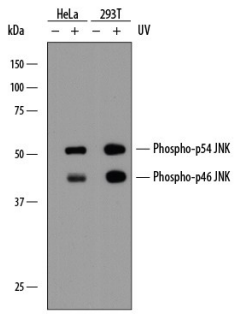
APPLICATIONS

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	10-35 µg/mL	See Below
Simple Western	10-25 µg/mL	See Below

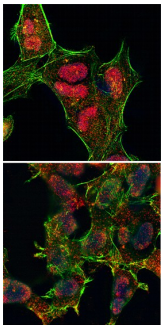
DATA

Western Blot



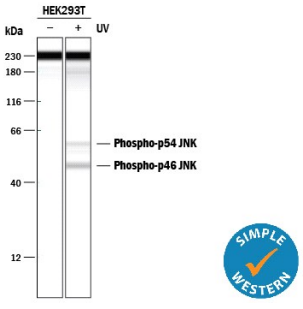
Detection of Human and Mouse Phospho-JNK (T183/Y185) by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and 293T human embryonic kidney cell line untreated (-) or treated (+) with 20 mJ/cm^2 ultraviolet light (UV) followed by a 30 minute recovery. PVDF membrane was probed with 1 µg/ml of Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Monoclonal Antibody (Catalog # MAB1205), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for Phospho-JNK (T183/Y185) at approximately 46 and 54 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Phospho-JNK (T183/Y185) in HEK293 Human Cell Line. JNK phosphorylated at T183/Y185 was detected in immersion fixed HEK293 human embryonic kidney cell line untreated (lower panel) or treated with UV radiation (upper panel) using Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Monoclonal Antibody (Catalog # MAB1205) at 25 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. Filamentous actin was stained with fluorescein-conjugated phalloidin (green). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human Phospho-JNK (T183/Y185) by Simple Western™. Simple Western lane view shows lysates of HEK293T human embryonic kidney cell line untreated (-) or treated (+) with 20 J/m^2 ultraviolet light (UV) followed by a 30 minute recovery, loaded at 0.2 mg/mL. A specific band was detected for Phospho-JNK (T183/Y185) at approximately 46 and 56 kDa (as indicated) using 20 µg/ml of Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Monoclonal Antibody (Catalog # MAB1205). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

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.

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BACKGROUND

The c-Jun N-terminal Kinases (JNKs) are part of the MAPK (mitogen-activated protein kinase) system that transmits signals from the extracellular milieu to both the cytoplasm and nucleus of the cell. Following perturbation at the cell membrane, MEKKs/MAP3Ks are initially activated, followed by their activation of MKKs/MAP2Ks, and MKKs activation of MAPKs/MAP(1)Ks. There are three classes of MAPKs: ERKs, p38 Kinases and JNKs. JNKs are 45-55 kDa protein products of three genes which, through alternative splicing, generate up to 10 possible isoforms. The phosphorylation targets for MAPKs vary, but include p53, c-MYC, ATF2 and c-Jun, the latter molecule representing the namesake for the enzyme group. The three human JNKs share approximately 80% aa sequence identity. JNKs from human, mouse and rat all contain a conserved Met-Met-Thr(183)-Pro-Tyr(185)-Val-Val motif that undergoes dual phosphorylation by MMK4 and MMK7 to activate the different JNKs. Activated by environmental stresses and inflammatory cytokines, JNKs translocate to the nucleus where they regulate the activity of several transcription factors; including the c-Jun component of AP-1 and ATF-2.

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