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

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1205

## 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** | Polyclonal Rabbit IgG |
| **Purification** | Antigen and protein A Affinity-purified |
| **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.<br>*Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS. |

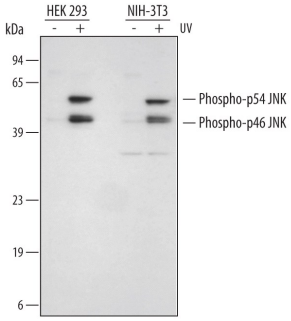
## APPLICATIONS

**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 |
| **Immunohistochemistry** | 5-15 µg/mL | See Below |
| **Simple Western** | 5 µg/mL | See Below |

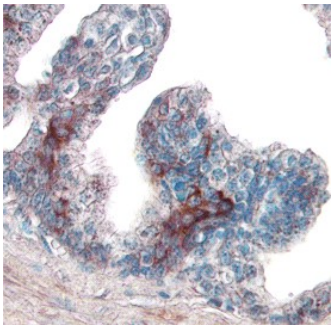
## DATA

### Western Blot



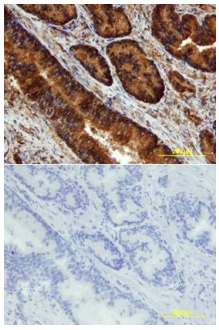

**Detection of Human and Mouse Phospho-JNK (T183/Y185) by Western Blot.** Western blot shows lysates of HEK293 human embryonic kidney cell line and NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 100 J/m$^2$ UV-C for 30 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1205), 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 6.

### Immunohistochemistry



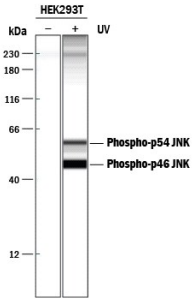
**JNK in Human Breast Cancer Tissue.** JNK phosphorylated at T183/Y185 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1205) at 15 µg/mL overnight at 4 °C. Tissue was stained using 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 immersion fixed paraffin-embedded Tissue Sections.

### Immunohistochemistry



**JNK in Human Prostate.** JNK phosphorylated at T183/Y185 was detected in immersion fixed paraffin-embedded sections of human prostate array using Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1205) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

### 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 100 J/m$^2$ ultraviolet light (UV) for 30 minutes, loaded at 0.2 mg/mL. Specific bands were detected for Phospho-JNK (T183/Y185) at approximately 56 and 46 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-JNK (T183/Y185) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1205). 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

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|---|---|
| **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.<br>*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.**<br>● 12 months from date of receipt, -20 to -70 °C as supplied.<br>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>● 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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