Human Phospho-RelA/NFκB p65 (S536) Antibody
Recombinant Monoclonal Rabbit IgG Clone # 1091B
Catalog Number: MAB72261

DESCRIPTION

Species Reactivity
Human

Specificity
Detects human RelA/NFκB p65 when phosphorylated at S536.

Source
Recombinant Monoclonal Rabbit IgG Clone # 1091B

Purification
Protein A or G purified from cell culture supernatant

Immunogen
Phosphopeptide containing the RelA/NFκB p65 S536 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
0.1 µg/mL
See Below
Immunocytochemistry
5-25 µg/mL
See Below
Simple Western
1 µg/mL
See Below

DATA

Western Blot
Detection of Human Phospho-RelA/NFκB p65 (S536) by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 100 nM Calyculin A (Catalog # 1336) and 20 ng/mL Recombinant Human TNF-α (Catalog # 210-TA) for 10 minutes. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human Phospho-RelA/NFκB p65 (S536) Monoclonal Antibody (Catalog # MAB72261) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-RelA/NFκB p65 (S536) at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western
Detection of Human Phospho-RelA/NFκB p65 (S536) by Simple Western. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 100 nM Calyculin A (Catalog # 1336) and 20 ng/mL Recombinant Human TNF-α (Catalog # 210-TA) for 10 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-RelA/NFκB p65 (S536) at approximately 66 kDa (as indicated) using 1.0 µg/mL of Rabbit Anti-Human Phospho-RelA/NFκB p65 (S536) Monoclonal Antibody (Catalog # MAB72261). 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.

Immunocytochemistry
Phospho-RelA/NFκB p65 (S536) in HT-29 Human Cell Line. RelA/NFκB p65 phosphorylated at S536 was detected in immersion fixed HT-29 human colon adenocarcinoma cell line untreated (lower panel) or treated with Calyculin A (Catalog # 1336) and Recombinant Human TNF-α (Catalog # 210-TA; upper panel) using Rabbit Anti-Human Phospho-RelA/NFκB p65 (S536) Monoclonal Antibody (Catalog # MAB72261) 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 and cell surfaces. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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

RelA belongs to a family of transcription factors, NFκB (Nuclear Factor kappa from B cells) complex, that play a fundamental role in inflammatory and immune responses. The NFκB complex is composed of a heterodimer of a Rel family member (RelA, c-Rel, RelB) and either NFκB1 or NFκB2 subunits. RelA and NFκB1 are the most common heterodimeric pair. The NFκB complex is sequestered in the cytoplasm by inhibitory IκB proteins. Upon cellular activation, the ubiquitin-proteasome pathway degrades the IκB proteins allowing the NFκB complex to translocate to the nucleus and activate gene transcription.