

DESCRIPTION

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| Species Reactivity | Human |
| Specificity | Detects human RelA/NFκB p65 when phosphorylated at S536. |
| Source | Monoclonal Mouse IgG ₁ Clone # 817403 |
| Purification | Protein A or G purified from hybridoma 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 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 | 2 μg/mL | See Below |
| Immunocytochemistry | 8-25 μg/mL | See Below |

DATA

Western Blot

Detection of Human Phospho-RelA/NFκB p65 (S536) by Western Blot.
Western blot shows lysates of HT-29 human colon adenocarcinoma cell line and HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 100 nM Calyculin A and 20 ng/mL Recombinant Human TNF-α (Catalog # 210-TA) for 10 minutes. PVDF membrane was probed with 2 μg/mL of Mouse Anti-Human Phospho-RelA/NFκB p65 (S536) Monoclonal Antibody (Catalog # MAB7226) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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 2.

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 and Recombinant Human TNF-α (Catalog # 210-TA; upper panel) using Mouse Anti-Human Phospho-RelA/NFκB p65 (S536) Monoclonal Antibody (Catalog # MAB7226) at 2 μg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) 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

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| Reconstitution | Sterile PBS to a final concentration of 0.5 mg/mL. |
| 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

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.