

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human FADD when phosphorylated on Ser194.
Source	Monoclonal Mouse IgG _{2A} Clone # 709736
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human FADD S194 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	8-25 µg/mL	See Below

DATA

<p>Western Blot</p> <p>Detection of Human Phospho-FADD (S194) 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 1 µg/mL nocodazole or 4 mM hydroxyurea for 20 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Phospho-FADD (S194) Monoclonal Antibody (Catalog # MAB7047) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for FADD at approximately 28 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p> <p>Phospho-FADD (S194) in HeLa Human Cell Line. FADD phosphorylated at S194 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line stimulated with nocodazole using Mouse Anti-Human Phospho-FADD (S194) Monoclonal Antibody (Catalog # MAB7047) at 10 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 nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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

Human FADD (Fas-Associating protein with Death Domain), also known as MORT1, is a 25 kDa, 208 amino acid (aa) adaptor protein that mediates signaling of death domain-containing TNF receptor superfamily members and is thus a major regulator of apoptosis. FADD is also implicated in proliferation, cell cycle progression, tumor development, inflammation, innate immunity and autophagy. Phosphorylation on human FADD Ser194 (Ser191 in mouse) is found in T cells arrested at G2/M in the cell cycle; this effect is independent of death domain receptors. Human FADD shares 68% aa identity with mouse and rat FADD.