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RABBIT ANTI-MOUSE HSP25 (pS^{86}) PHOSPHOSPECIFIC POLYCLONAL ANTIBODY

CATALOG NUMBER: AB3563 **QUANTITY:** 100 µL

LOT NUMBER:

BACKGROUND:

Heat Shock Protein 25 (HSP25), is a 25 kDa member of a family of proteins whose expression and function are stimulated by heat shock and other stress stimuli. A major function of these proteins is to serve as chaperones that bind to and stabilize the active conformation of other proteins. HSP25, along with other members of the small HSP group, possesses a C-terminal alpha-crystalline homology domain. HSP25 is localized to the cytoplasm of unstressed cells but can redistribute to the nucleus in response to stress, where it may function to stabilize DNA and/or the nuclear membrane. Cytoplasmic HSP25 exists in multiple complexes. One complex consists of HSP25, Akt (PKB), MAPKAP-kinase 2, and p38 MAPK. The presence of HSP25 in this complex is required for Akt activation by stress stimuli. Another complex consists of HSP25 and the IKK complex. HSP25 is also an actin capping protein that binds to the barbed (growing) ends of actin filaments, thereby inhibiting filament extension. Phosphorylation of HSP25 on serine 86 by MAPKAP-kinase 2 leads to HSP25 dissociation from the Akt/MAPKAP-kinase 2/p38 MAPK complex and from actin filaments, and stimulates HSP25 binding to the IKK complex.

IMMUNOGEN:

The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of mouse HSP25 that contains serine 86.

APPLICATIONS:

For Western blot applications, we recommend using the antibody at a 1:1,000 starting dilution. Optimal working dilutions must be determined by end user.

Peptide Competition

Lysates prepared from NIH3T3 cells left unstimulated (1) or treated with anisomycin (2-6) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were left untreated (1-5) or treated with lambda (λ) phosphatase (6), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with HSP25 [pS^{86}] (mouse) antibody for one hour at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2, 6), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate in 3% BSA-TBST buffer, and bands were detected using standard methods.

The data show that only the peptide corresponding to HSP25 [pS^{86}] (mouse) blocks the antibody signal, thereby demonstrating the specificity of the antibody. The signal was completely removed by phosphatase treatment demonstrating that the antibody interacts specifically with the phosphorylated protein.

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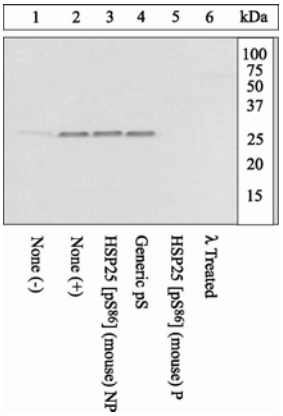

SPECIES REACTIVITY: Mouse HSP25. Endogenous human HSP27 phosphorylated at serine 82 (HeLa cells treated with TNF-□) was weakly detected by this antibody.

CONTROL: NIH3T3 cells treated with anisomycin.

FORMAT: Purified from rabbit serum by epitope-specific affinity chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated HSP25 (the mouse homolog of human HSP27). The final product is generated by affinity chromatography using an HSP25-derived peptide that is phosphorylated at serine 86.

PRESENTATION: Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier. 0.05% sodium azide

STORAGE/HANDLING: Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

REFERENCES:

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