Human Phospho-HSP27 (S78/S82) Antibody

Recombinant Monoclonal Rabbit IgG Clone # 1026B
Catalog Number: MAB23141

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HSP27 when dually phosphorylated at S78/S82 in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1026B
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Phosphopeptide containing the human HSP27 S78/S82 site Accession # P04792
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.

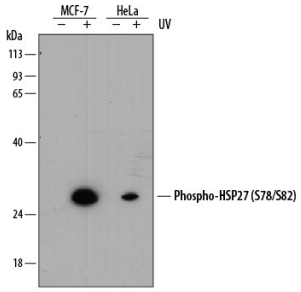
APPLICATIONS

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	5-25 µg/mL	See Below

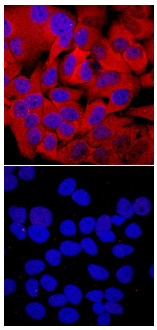
DATA

Western Blot



Detection of Human Phospho-HSP27 (S78/S82) by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line and HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 20 mJ/cm^{2}ultraviolet light (UV) with a 30 minute recovery. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human Phospho-HSP27 (S78/S82) Monoclonal Antibody (Catalog # MAB23141) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). A specific band was detected for Phospho-HSP27 (S78/S82) at approximately 27 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Phospho-HSP27 (S78/S82) in HeLa Human Cell Line. HSP27 phosphorylated at S78/S82 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line unstimulated (lower panel) or stimulated with 20 mJ/cm^{2}ultraviolet radiation (upper panel) using Rabbit Anti-Human Phospho-HSP27 (S78/S82) Monoclonal Antibody (Catalog # MAB23141) at a 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and nuclei. 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

Heat shock proteins (HSPs) are a family of highly conserved stress response proteins. Heat shock proteins function primarily as molecular chaperones by facilitating the folding of other cellular proteins, preventing protein aggregation or targeting improperly folded proteins to specific degradative pathways. HSPs are typically expressed at low levels under normal physiological conditions but are dramatically up-regulated in response to cellular stress. Elevated levels of HSPs have been observed in association with ischemia/reperfusion, cancer, and chronic heart failure. HSP27 is a member of the small heat shock protein family, which also includes HSP25 and the α-crystallins. Ser78 and Ser82 of HSP27 are phosphorylated in vivo in response to growth factors or heat shock, and the extent of phosphorylation plays a role in determining specific functions. HSP27 forms a large oligomer and functions as an anti-apoptotic molecule, regulating apoptosis through direct interaction with key components of the apoptotic pathway. HSP27 binds and sequesters cytochrome c released from the mitochondria in response to an apoptotic stimulus. This prevents the proper assembly of the apoptosome and subsequently, the activation of procaspase-9 and procaspase-3.

References:

1. Gusev, N.B. *et al.* (2002) Biochemistry (Moscow) **67**:511.
2. Garrido, C. *et al.* (2001) Biochem. Biophys. Res. Commun. **286**:433.
3. Garrido, C. (2002) Cell Death Diffr. **9**:483.
4. Brvey, J-M. *et al.* (2000) Nat. Cell Biol. **2**:645.

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