

HSP70 Antibody

HSP70 Antibody, Clone 1H11 Catalog # ASM10169

Specification

HSP70 Antibody - Product Information

Application WB
Primary Accession PODMV8
Other Accession NP_005336.3
Host Mouse
Isotype IgG1

Reactivity Human, Mouse, Rat, Pig, Bovine

Clonality Monoclonal Format ATTO 390

Description

Mouse Anti-Human Cell Membrane HSP70 Monoclonal IgG1

Target/Specificity

Binds to cell surface HSP70 without permeabilization of cell membrane in tumor cell lines. Does not bind to intracellular HSP70 at low concentrations. Detects a ~70 kDa band in Western Blot.

Other Names

HSP70 1 Antibody, HSP70 2 Antibody, HSP70.1 Antibody, HSP72 Antibody, HSPA1 Antibody, HSPA1B Antibody

Immunogen

Human native HSP70 protein

Purification

Protein G Purified

Storage -20°C

Storage Buffer

PBS pH 7.4, 50% glycerol, 0.9% Sodium Azide

Shipping Temperature

Certificate of Analysis

A 1:250 dilution of SMC-249 was sufficient for detection of cell surface HSP70 in HCT116 cells using Fluorescence-activated cell sorting, applied in flow cytometry, with FITC as the fluorescent probe.

Blue Ice or 4ºC

Cellular Localization

Cell membrane

HSP70 Antibody - Protocols

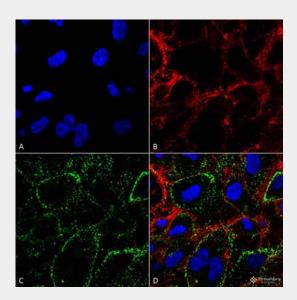
Provided below are standard protocols that you may find useful for product applications.

Western Blot

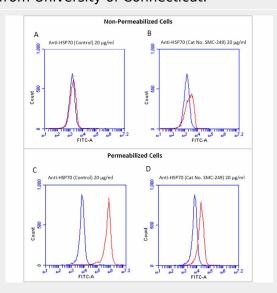


- Blocking Peptides
- Dot Blot
- <u>Immunohistochemistry</u>
- Immunofluorescence
- <u>Immunoprecipitation</u>
- Flow Cytomety
- Cell Culture

HSP70 Antibody - Images



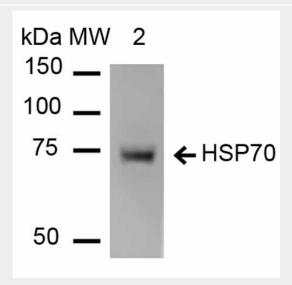
Immunocytochemistry/Immunofluorescence analysis on non-permeabilized HCT116 cells using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11: FITC conjugate (ASM10169D-FITC) showing cell membrane staining. Tissue: HCT116 cells. Species: Human. Fixation: 4% Formaldehyde. Permeabilization: None. Primary Antibody: Mouse Anti-HSP70 Monoclonal Antibody (ASM10169) at 1:100. Counterstain: Wheat germ agglutinin Texas red membrane marker; DAPI (blue) nuclear stain. Localization: Cell surface, cell membrane. (A) DAPI nuclear stain. (B) Wheat germ agglutinin Texas red. (C) HSP70 Antibody. (D) Composite. Courtesy of: Lawrence Hightower, Charles Giardina, and Didem Ozcan from University of Connecticut.



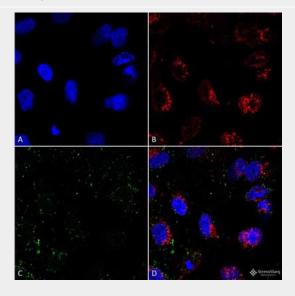
Fluorescence-activated cell sorting (FACS) using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11: FITC conjugate (ASM10169D-FITC). Tissue: Jurkat E6.1. Species: Human.



Unfixed/non-permeabilized cells were incubated with (A) Anti-HSP70 control antibody, or (B) Anti-HSP70 antibody, clone 1H11 (ASM10169D-FITC), for 40 min at 4°C. Fixed/permeabilized cells were incubated with (C) Anti-HSP70 control antibody, or (D) Anti-HSP70 antibody, clone 1H11 (ASM10169D-FITC) for 40 min at 4°C. Analysis shows that Anti-HSP70 (ASM10169) can detect low levels of cell surface HSP70, and can faintly detect intracellular HSP70. Courtesy of: Dr. Elyse Ireland, Institute of Medicine, University of Chester.

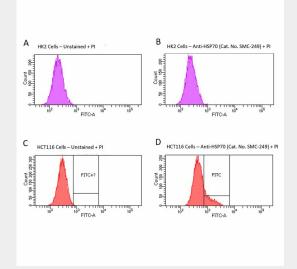


Western Blot analysis of Human Heat shocked HeLa cell lysates showing detection of HSP70 protein using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11 (ASM10169). Lane 1: Molecular Weight ladder (MW). Lane 2: HeLa cell lysates. Load: 20 µg. Primary Antibody: Mouse Anti-HSP70 Monoclonal Antibody (ASM10169) at 1:1000.

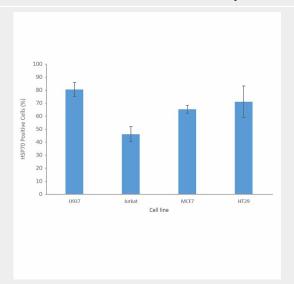


Immunocytochemistry/Immunofluorescence analysis on permeabilized HCT116 cells using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11: FITC conjugate (ASM10169D-FITC) showing faint cell membrane and intracellular staining. Tissue: HCT116 cells. Species: Human. Fixation: 4% Formaldehyde. Permeabilization: 0.2% PBST. Primary Antibody: Mouse Anti-HSP70 Monoclonal Antibody (ASM10169) at 1:100. Counterstain: Wheat germ agglutinin Texas red membrane marker; DAPI (blue) nuclear stain. Localization: Cell surface, faint intracellular. (A) DAPI nuclear stain. (B) Wheat germ agglutinin Texas red. (C) HSP70 Antibody. (D) Composite. Courtesy of: Lawrence Hightower, Charles Giardina, and Didem Ozcan from University of Connecticut.





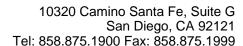
Fluorescence-activated cell sorting analysis using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11: FITC conjugate (ASM10169D-FITC). Tissue: HCT116 and HK2 cells. Species: Human. Primary Antibody: Mouse Anti-HSP70 Monoclonal Antibody (ASM10169) at 1:100 for 90 min at 4°C. Counterstain: Propidium Iodide nuclear stain. (A) HK2 cells unstained. (B) HK2 cells and HSP70 Antibody. (C) HCT116 cells unstained. (D) HCT116 cells and HSP70 Antibody. Shows that HSP70 Antibody binds to the cell surface of tumor cells but not non-tumor cells. Courtesy of: Lawrence Hightower, Charles Giardina, and Didem Ozcan from University of Connecticut.



Fluorescence-activated cell sorting analysis using Mouse Anti-HSP70 Monoclonal Antibody, Clone 1H11: FITC conjugate (ASM10169D-FITC). Tissue: Jurkat E6.1, U937, MCF7 and HT29 cells . Species: Human. Fixation: No fixation. Permeabilization: None. Primary Antibody: Mouse Anti-HSP70 Monoclonal Antibody (ASM10169) at 20 $\mu g/ml$ for 40 min at 4°C. Counterstain: Propidium Iodide nuclear stain at 2.5 $\mu g/ml$ for 5 min at 4°C. Shows that binding of HSP70 Antibody is not cell-line specific. Courtesy of: Dr. Elyse Ireland, Institute of Medicine, University of Chester.

HSP70 Antibody - Background

HSP70 is a highly conserved protein that is ubiquitously expressed. It can be found in chloroplasts, eukaryotic cytosol, endoplasmic reticulum, and mitochondria, but also embedded in the cell membrane and in the extracellular space (1,2,3,4). Even though HSP70 is one of the most studied heat shock proteins, the export mechanism and method of membrane insertion are not fully





understood. The majority of the proteins in the heat shock family lack a consensus signal for secretion via the ER-Golgi pathway (5). Researchers have found that HSP70 may be released from cells via exosomes or secretory vesicles (6).

Although HSP70 is ubiquitously expressed, there is not much information about its presence on cell surface. The finding that HSP70 is localized on the cell surface of cancer cells but not normal cells suggests a conformational change of HSP70 in the lower pH environment characteristic of cancer cells (7). The presence of cell membrane embedded HSP70 has been found to increase the stability of cancer cells, thereby protecting tumors from environmental stress (8, 9).

Anti-HSP70 antibody, clone 1H11 (Catalog No. SMC-249) is unique from other commercially available antibodies in that it can bind to the extracellular region of the cell membrane embedded HSP70 protein, allowing researchers to differentiate between membrane bound and intracellular HSP70 across cancer cells types.

HSP70 Antibody - References

- 1. Gribaldo, S. et al. (1999) J. Bacteriol. 181, 434-443.
- 2. Sharma, D. & Masison, D.C. (2009) Protein Pept. Lett. 16, 571-581.
- 3. Sharma, D. et al. (2009) PLoS. ONE.4, e6644.
- 4. Kampinga, H.H. & Craig, E.A. (2010) Nat. Rev. Mol. Cell Biol. 11, 579-592.
- 5. Nickel, W. & Seedorf, M. (2008) Annu. Rev. Cell Dev. Biol. 24, 287-308.
- 6. Multhoff, G. & Hightower, L.E. (1996) Cell Stress. Chaperones. 1, 167-176.
- 7. De Maio, A. (2011) Cell Stress. Chaperones. 16, 235-249.
- 8. Horvath, I. & Vigh, L. (2010) Nature. 463, 436-438.
- 9. Horvath, I., Multhoff, G., Sonnleitner, A., & Vigh, L. (2008) Biochim. Biophys. Acta 1778, 1653-1664.