abcam

Product datasheet

Anti-Hsp70 antibody [5A5] ab2787

★★★★★ 18 Abreviews 174 References 14 Images

Overview

Product name	Anti-Hsp70 antibody [5A5]
Description	Mouse monoclonal [5A5] to Hsp70
Host species	Mouse
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, IP, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Dog, Human, African green monkey
Immunogen	Recombinant fragment within Human Hsp70 aa 122-264 (N terminal). The exact sequence is proprietary.
Epitope	Epitope mapping with a panel of HSP 70 deletion mutants suggests that the epitope recognized is located between amino acids 122-264 of human HSP 70, a region that has been shown to be involved in ATP binding. This is the first monoclonal antibody reported to react with: 1) The ATP binding region of HSP 70. 2) An epitope in the amino terminus of HSP 70.
Positive control	WB: HEK-293, A549, IMR-32, Jurkat, Jurkat treated with heat shock , MDCK, NIH/3T3, PC-12, COS-7, HeLa, K562, A431, U-2 OS whole cell and mouse ovary tissue lysate. IHC-P: Human prostate, tonsil and testis tissue. ICC: HeLa, NIH- 3T3, U251 MG, C6 cells. IP: HeLa cell lysates.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS, BSA
Purity	Protein A purified
Primary antibody notes	The HSP 70 family is a set of highly conserved proteins that are induced by a variety of biological

stresses, including heat stress, in every organism in which the proteins have been examined. The human HSP 70 family members include: HSP 70, a protein which is strongly inducible in all organisms but which is also constitutively expressed in primate cells; HSP 72, a 72 kDa protein that is induced exclusively under stress conditions; HSC 70, or cognate protein, is a 72 kDa, constitutively expressed, protein which is involved in the uncoating of clathrin coated vesicles; GRP 78, or BiP, is a glucose regulated 78 kDa protein localized in the endoplasmic reticulum; and p75, or HSP 75, a 75 kDa protein that is found within the mitochondria.

Clonality Monoclonal

Clone number 5A5

Isotype IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab2787 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

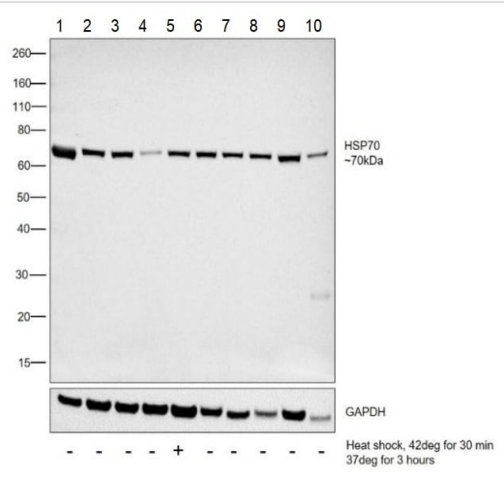
Application	Abreviews	Notes
IHC-P	★★★★★ **(2)**	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★★ **(11)**	1/1000. Detects proteins from ~70 kDa to ~78 kDa representing different members of the HSP 70 family. 2-dimensional gel electrophoresis is required to resolve the heat induced form of these proteins from their constitutively expressed counterparts.
ICC/IF	★★★★★ **(3)**	1/50 - 1/100.
IP	★★★★★ **(1)**	Use at 1-10 µg/mg of lysate. See Balashova et al.
Flow Cyt		1/100. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Relevance Function: In cooperation with other chaperones, the Hsp70 family stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage. In case of rotavirus A infection, serves as a post-attachment receptor for the virus to facilitate entry into the cell. Tissue specificity: HSPA1B is testis-specific.

Cellular localization Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Images



Western blot - Anti-Hsp70 antibody [5A5] (ab2787)

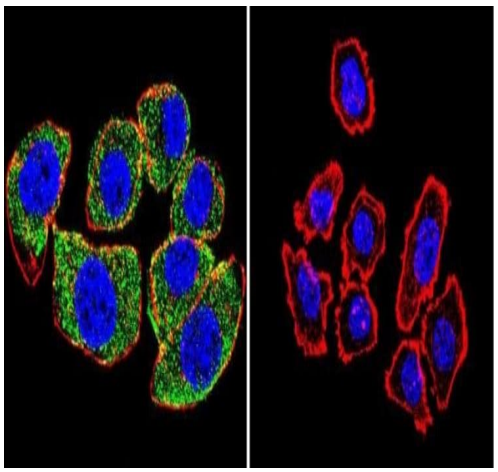
All lanes : Anti-Hsp70 antibody [5A5] (ab2787) at 1/500 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : IMR32 (Human brain neuroblast cell line) whole cell lysate
Lanes 4-5 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 6 : MDCK (Canine kidney cell line) whole cell lysate
Lane 7 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate
Lane 8 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 9 : COS-7 (African green monkey kidney fibroblast-like cell line) whole cell lysate
Lane 10 : Mouse ovary tissue lysate

Lysates/proteins at 30 µg per lane.

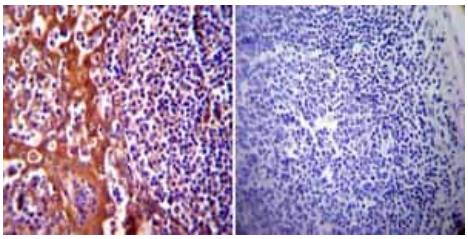
Secondary
All lanes : Goat anti-mouse IgG (H+L) HRP at 1/4000 dilution

Developed using the ECL technique.



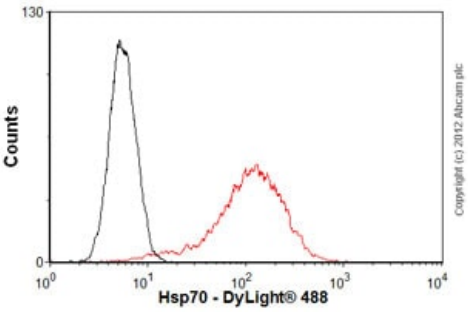
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [5A5] (ab2787)

Immunofluorescent analysis of U-251 MG (Human brain glioma cell line) cells labeling Hsp70 (green) with ab2787. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Heat Shock Protein 70 ab2787 at a dilution of 1/100-1/200 over night at 4^{o}C washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



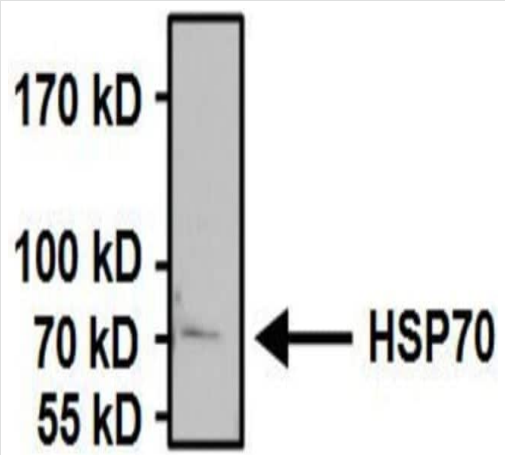
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [5A5] (ab2787)

Immunohistochemistry was performed on normal biopsies of deparaffinized Human tonsil tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (**ab2787**) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



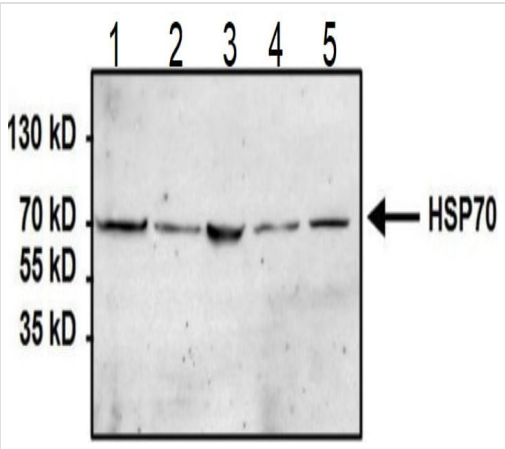
Flow Cytometry - Anti-Hsp70 antibody [5A5] (ab2787)

Overlay histogram showing Jurkat cells stained with ab2787 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2787, 1:100 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1:500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunoprecipitation - Anti-Hsp70 antibody [5A5] (ab2787)

Immunoprecipitation of Hsp70 was performed on HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate. Antigen:antibody complexes were formed by incubating 500µg whole cell lysate with 2µg of ab2787 overnight on a rocking platform at 4°C. The immune complexes were captured on 50µl Protein A/G Agarose, washed extensively, and eluted with buffer. Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was incubated with ab2787 (1:1000) overnight rotating at 4°C, washed in TBST, and probed with IP detection reagent-HRP at a dilution of 1:1000 for at least one hour. Chemiluminescent detection was performed.



Western blot - Anti-Hsp70 antibody [5A5] (ab2787)

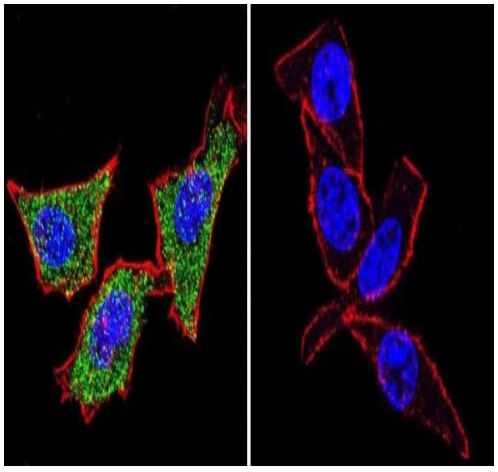
All lanes : Anti-Hsp70 antibody [5A5] (ab2787) at 1/1000 dilution

Lane 1 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate
Lane 4 : A431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 5 : U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate

Lysates/proteins at 50 µg per lane.

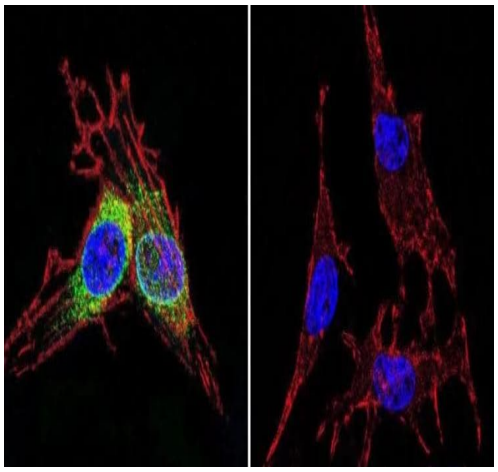
Secondary
All lanes : Goat anti-mouse IgG HRP secondary antibody at 1/20000 dilution

Western blot analysis of Hsp70 was performed by 15µl of prestained protein ladder onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was incubated overnight at 4°C on a rocking platform, washed in TBS-0.1%Tween 20, and incubated with secondary antibody for at least 1 hour. Chemiluminescent detection was performed.



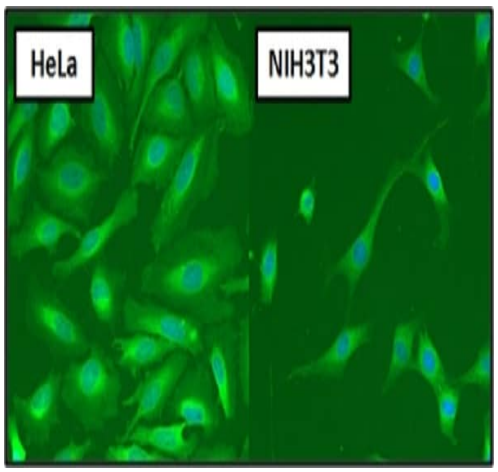
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [5A5] (ab2787)

Immunofluorescent analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Hsp70 (green) with ab2787. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Heat Shock Protein 70 ab2787 at a dilution of 1/100-1/200 over night at 4^{O}C washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



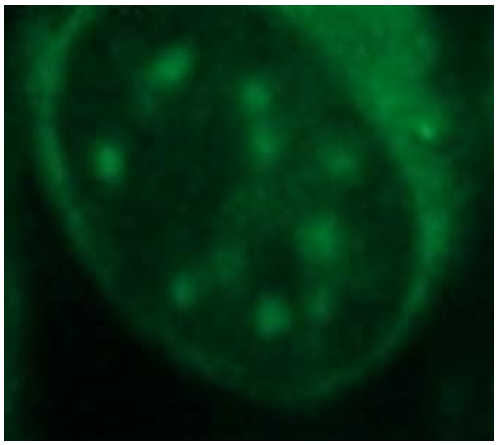
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [5A5] (ab2787)

Immunofluorescent analysis of C6 (Rat glial tumor cell line) cells labeling Hsp70 (green) with ab2787. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (right) or with or an antibody recognizing Heat Shock Protein 70 (ab2787) (left) at a dilution of 1/100-1/200 over night at 4^{O}C washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [5A5] (ab2787)

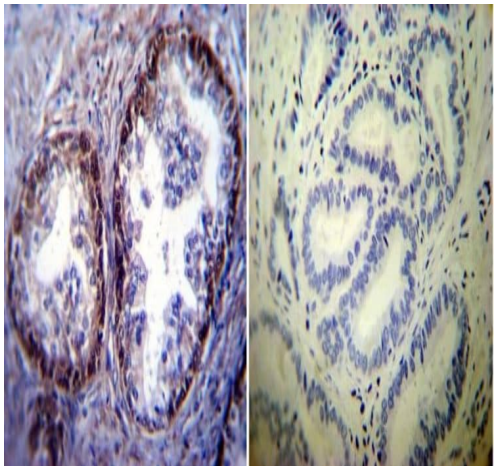
Immunofluorescent analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) and NIH/3T3 (Mouse embryo fibroblast cell line) cells labeling Hsp70 (green) with ab2787. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with a HSP70 Monoclonal Antibody, at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-mouse IgG secondary antibody at a dilution of 1/400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [5A5] (ab2787)

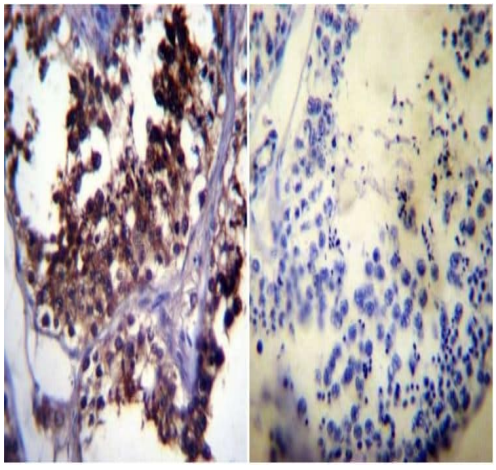
Image from Park R et al, J Biol Chem. 2011 Mar 18;286(11):9748-62. Epub 2011 Jan 13, Fig 4. DOI 10.1074/jbc.M110.198325

ab2787 staining Hsp70 in 2089 cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed in methanol for 30 minutes at -20°C, washed with PBS, and incubated in blocking solution (10% human serum in PBS) for 1 hour at room temperature. Cells were stained with ab2787 diluted in blocking solution for 1 hour at room temperature in humidified chambers. Cells were washed with PBS and then incubated with secondary antibody diluted 1/200 in blocking solution for 1 hour at room temperature in opaque humidified chambers.



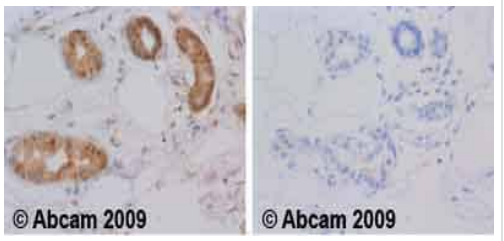
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [5A5] (ab2787)

Immunohistochemistry was performed on cancer biopsies of deparaffinized Human prostate carcinoma tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/100 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (**ab2787**) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



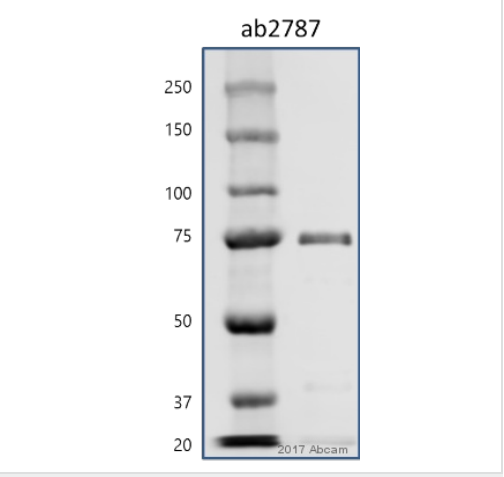
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [5A5] (ab2787)

Immunohistochemistry was performed on normal biopsies of deparaffinized Human testis tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (**ab2787**) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [5A5] (ab2787)

ab2787 staining human skin. Staining is localized to the cytoplasm and nucleus.
Left panel: with primary antibody at 1/100. Right panel: isotype control.
Sections were stained using an automated system at room temperature. Sections were rehydrated and antigen retrieved. Slides were peroxidase blocked in 3% H_2O_2 in methanol for 10 minutes. They were then blocked for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Western blot - Anti-Hsp70 antibody [5A5] (ab2787)
This image is courtesy of an anonymous Abreview

Western blot analysis of U2OS cell lysate (30µg/lane) labelling Hsp70 with ab2787 at 1/1000 in 5% milk in TBST for 13 hours at 4°C. A IRDye® 800-conjugated Goat anti-Mouse polyclonal (1/10000) was used as the secondary antibody.

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