


Product datasheet

Anti-Hsp70 antibody [2A4] ab5442

★★★★★ 1 Abreviews 16 References 15 Images

Overview

Product name	Anti-Hsp70 antibody [2A4]
Description	Mouse monoclonal [2A4] to Hsp70
Host species	Mouse
Specificity	ab5442 detects several members of the heat shock protein 70 kDa (Hsp 70) gene family including Hsp 70, Hsc 70 and, following heat shock, Hsp 72 from yeast, Drosophila, fish, mouse, avian, amphibian and human samples. Immunofluorescence staining of Hsp 70 in heat shocked HeLa cells with ab5442 results in cytoplasmic staining.
Tested applications	Suitable for: Flow Cyt, WB, IP, ICC/IF, IHC-P
Species reactivity	Reacts with: Mouse, Human, Saccharomyces cerevisiae Predicted to work with: Cow, Pig 
Immunogen	Recombinant fragment corresponding to Human Hsp70. Database link: P0DMV8
Epitope	Epitope mapping with a panel of Hsp 70 deletion mutants suggests that the epitope recognized is located between amino acids 437-479 of human Hsp 70.
Positive control	ICC: heat shocked HeLa cells; WB: HeLa, HEK293T, HepG2, A-431, K-562, and MCF7 whole cell lysates.
General notes	<p>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.</p> <p>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</p>

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: 99% PBS

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; GRP78, 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	2A4
Isotype	IgM

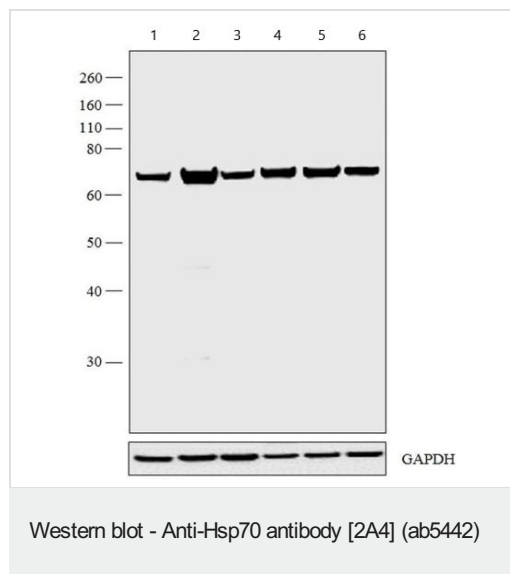
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5442 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
Flow Cyt		Use 1µg for 10 ⁶ cells. ab91545 - Mouse monoclonal IgM, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 70 kDa (predicted molecular weight: 70 kDa).
IP		Use a concentration of 2 µg/ml.
ICC/IF		1/100 - 1/200.
IHC-P		1/200. Antigen retrieval is not essential but may optimise staining (using a heat mediated method with citrate buffer).

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.



All lanes : Anti-Hsp70 antibody [2A4] (ab5442) at 1 µg/ml

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 4 : A431 (human epidermoid carcinoma cell line) whole cell lysate

Lane 5 : K562 (human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 6 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate

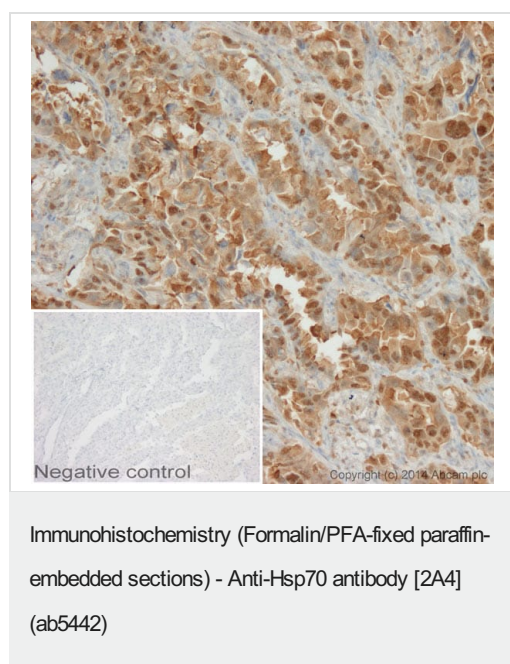
Lysates/proteins at 30 µg per lane.

Secondary

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

Predicted band size: 70 kDa

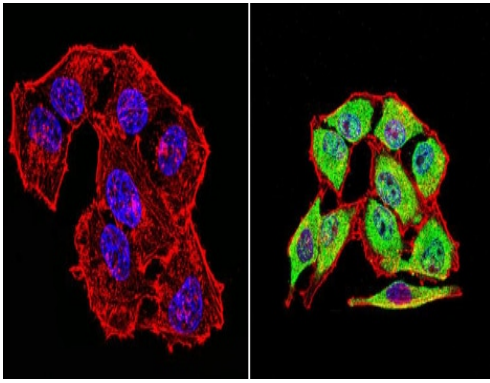
Observed band size: 70 kDa



IHC image of Hsp70 staining in human lung formalin fixed paraffin embedded tissue section*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab5442, 1/2000 dilution overnight at +4°C. An HRP-conjugated secondary ([ab97230](#), 1/2000 dilution) was used for 1hr at room temperature. The section was counterstained with haematoxylin and mounted with DPX.

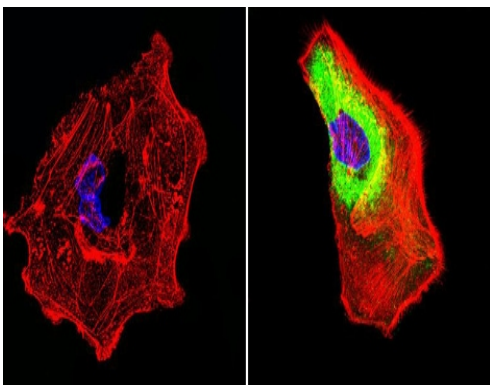
The inset negative control image is secondary-only at 1/500 dilution.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



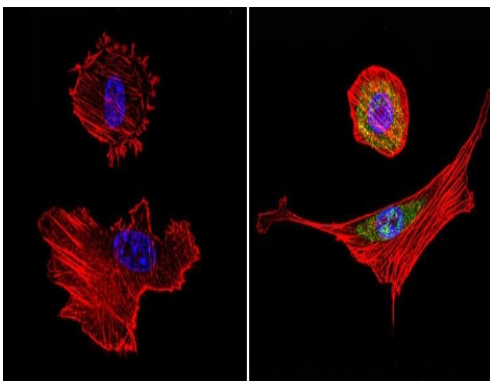
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in Hela cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:100 and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.



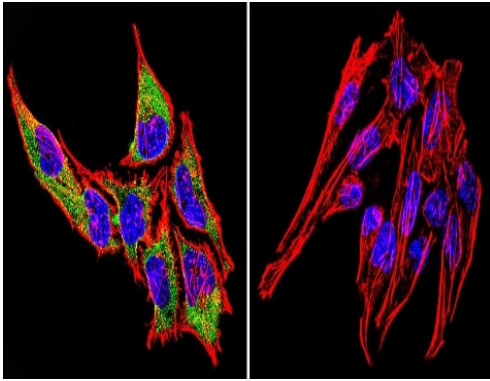
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in A431 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:100 and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.



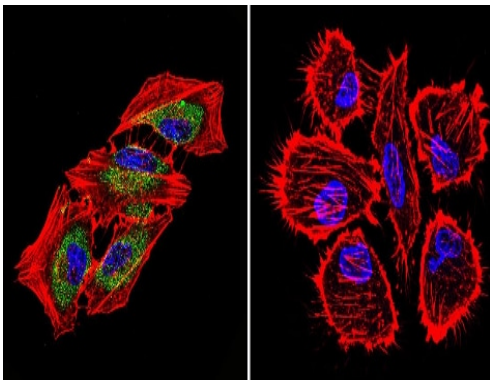
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in NIH-3T3 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:200 and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.



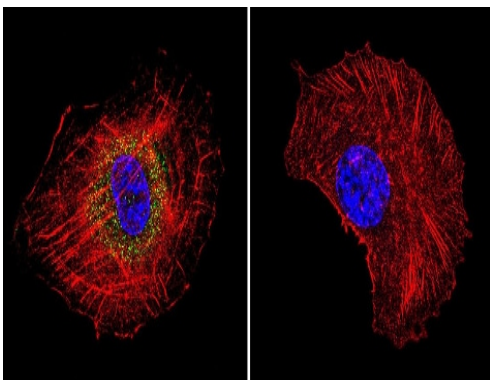
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab5442 at a dilution of 1:200 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



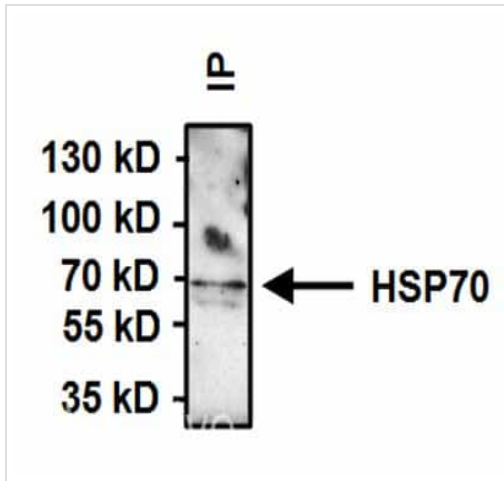
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 in NCI-H1299 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5442 at a dilution of 1:100 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



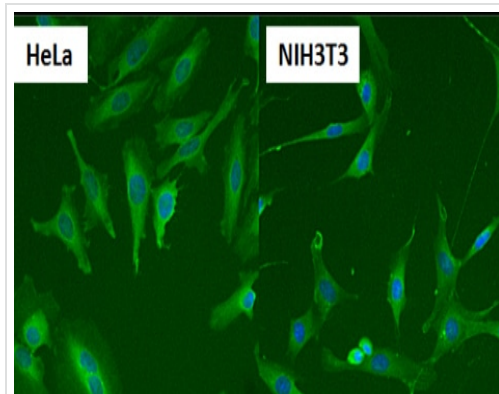
Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5442 at a dilution of 1:100 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



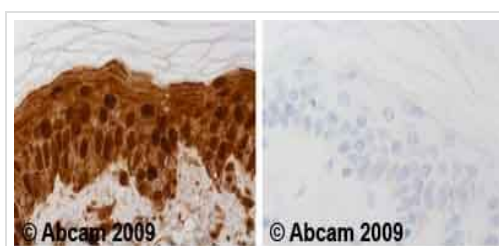
Immunoprecipitation - Anti-Hsp70 antibody [2A4]
(ab5442)

Immunoprecipitation of Hsp70 was performed on HeLa cells. Antigen-antibody complexes were formed by incubating 500ug of whole cell lysate with 2ug of HSP70 monoclonal antibody (ab5442) overnight on a rocking platform at 4°C. The immune complexes were captured on 50ul Protein A/G Agarose 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 probed with a Hsp70 monoclonal antibody (ab5442) at a dilution of 1:1000 overnight rotating at 4°C then washed in TBST and probed with a goat anti-mouse IgM secondary antibody at a dilution of 1:20000 for at least 1 hour. Chemiluminescent detection was performed.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in HeLa and NIH3T3 cells. 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 ab5442 at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with fluorescently labeled goat anti-mouse IgM secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



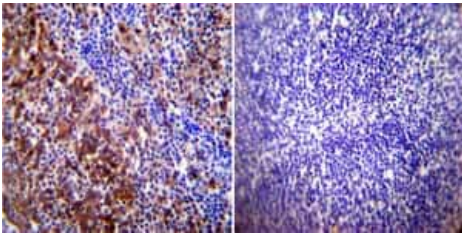
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4]
(ab5442)

Ab5442 staining human normal skin. Staining is localised to the cytoplasm and nucleus.

Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.

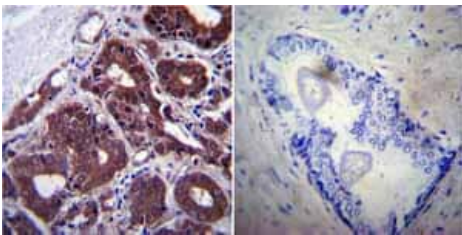
Sections were stained using an automated system DAKO Autostainer Plus, at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H₂O₂ in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 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



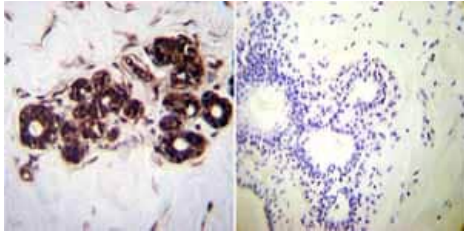
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

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 ab5442 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.



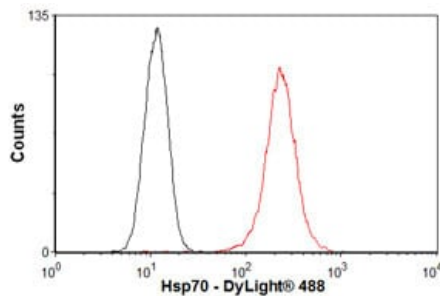
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

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 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 ab5442 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 [2A4] (ab5442)

Immunohistochemistry was performed on normal biopsies of deparaffinized Human breast 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 ab5442 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 [2A4] (ab5442)

Overlay histogram showing Jurkat cells stained with ab5442 (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 (ab5442, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgM (mu chain) (**ab97007**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgM [ICIGM] (**ab91545**, 2µg/1x10⁶ 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 (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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