abcam

Product datasheet

Anti-HLA A antibody [EP1395Y] - BSA and Azide free ab216653

KO VALIDATED Recombinant RabMAb

21 References 12 Images

Overview

Product name	Anti-HLA A antibody [EP1395Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1395Y] to HLA A - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human tonsil tissue. ICC/IF: MCF7 and Raji cells. WB: A431, Jurkat, THP-1, A549, HL-60 and Raji cell lysates. IP: THP-1 and A549 cell lysates. Flow Cyt (intra): Raji cells.
General notes	ab216653 is the carrier-free version of **ab52922**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1395Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab216653 in the following tested applications.

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

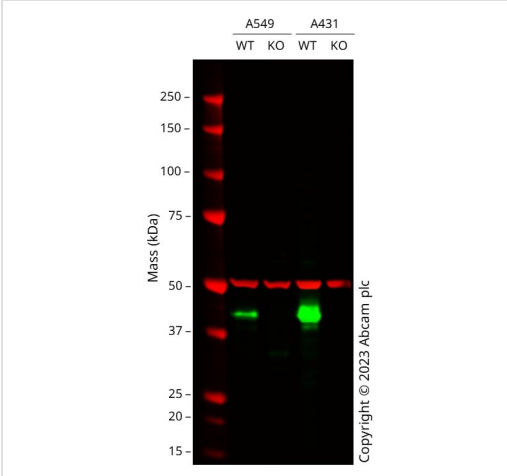
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Predicted molecular weight: 41 kDa.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Relevance HLA-A belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. Class I molecules play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. They are expressed in nearly all cells. The heavy chain is approximately 45 kDa and its gene contains 8 exons. Exon 1 encodes the leader peptide, exons 2 and 3 encode the alpha1 and alpha2 domains, which both bind the peptide, exon 4 encodes the alpha3 domain, exon 5 encodes the transmembrane region, and

exons 6 and 7 encode the cytoplasmic tail. Polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class one molecule. Typing for these polymorphisms is routinely done for bone marrow and kidney transplantation. Hundreds of HLA-A alleles have been described.

Images



Western blot - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

All lanes : Anti-HLA A antibody [EP1395Y] (**ab52922**) at 1/10000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : HLA-A knockout A549 cell lysate
Lane 3 : Wild-type A431 cell lysate
Lane 4 : HLA-A knockout A431 cell lysate

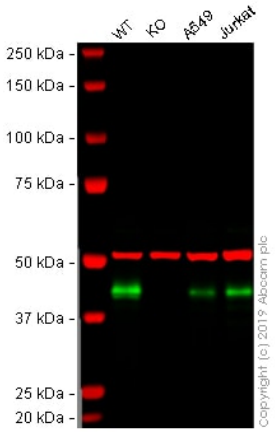
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 41 kDa
Observed band size: 41 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab52922**).

Anti-HLA-A antibody [EP1395Y] (**ab52922**) staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab52922** was shown to bind specifically to HLA-A. A band was observed at 41 kDa in wild-type A549 cell lysates with no signal observed at this size in HLA-A knockout cell line. To generate this image, wild-type and HLA-A knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

All lanes : Anti-HLA A antibody [EP1395Y] (**ab52922**) at 1/10000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : HLA A knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

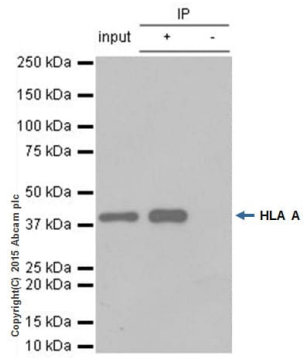
Performed under reducing conditions.

Predicted band size: 41 kDa
Observed band size: 40 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab52922**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab52922** observed at 40 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab52922 was shown to react with HLA-A in A431 wild-type cells in Western blot. Loss of signal was observed when HLA-A knockout sample was used. A431 wild-type and HLA-A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with **ab52922** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



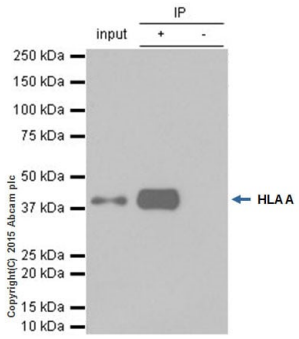
Immunoprecipitation - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

ab52922 (purified) at 1/20 immunoprecipitating HLA A in A549 whole cell lysate. 10 ug of cell lysate was present in the input. For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution. A rabbit monoclonal IgG (**ab172730**) was used intead of **ab128913** as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



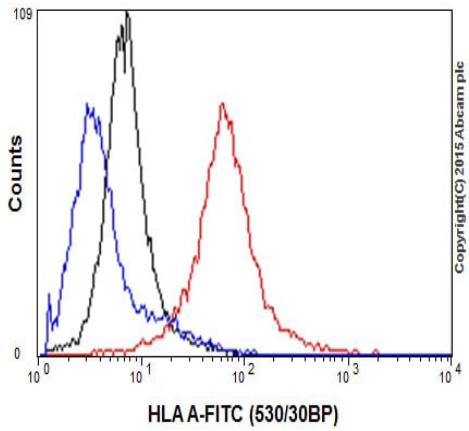
Immunoprecipitation - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

ab52922 (purified) at 1/20 immunoprecipitating HLA A in THP-1 whole cell lysate. 10 ug of cell lysate was present in the input. For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution. A rabbit monoclonal IgG (**ab172730**) was used intead of **ab128913** as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

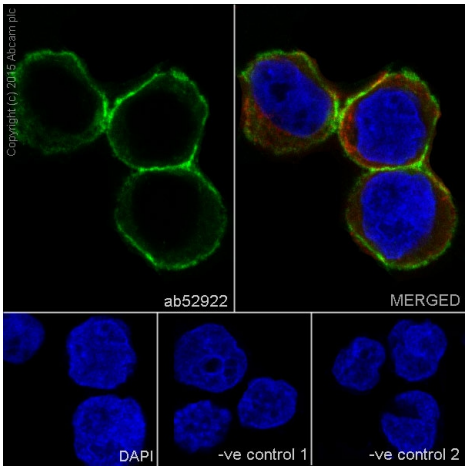
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Flow Cytometry (Intracellular) - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

Intracellular Flow Cytometry analysis of Raji cells labelling HLA A with purified **ab52922** at 1/40 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



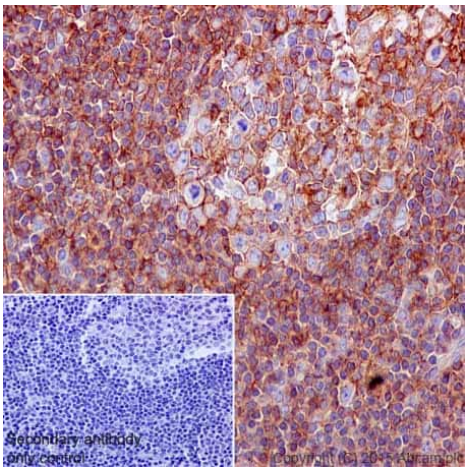
Immunocytochemistry/ Immunofluorescence - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

Immunocytochemistry/Immunofluorescence analysis of Raji (human Burkitt's lymphoma) cells labelling HLA A with purified **ab52922** at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

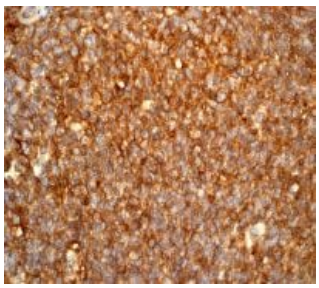
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

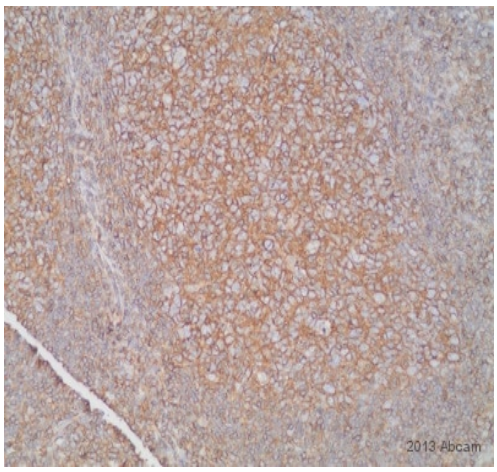
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling HLA A with purified **ab52922** at 1/100. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. **ab97051**, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

Ab52922 at 1/250 dilution staining human tonsil; paraffin embedded.

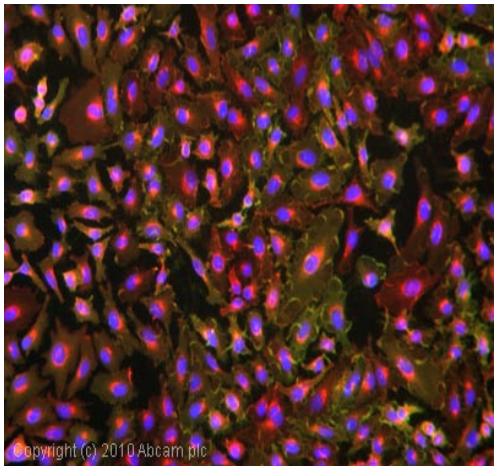
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)
This image is courtesy of an anonymous Abreview.

ab52922 staining HLA A in Human tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% H_2O_2 for 10 minutes at 25°C; antigen retrieval was by heat mediation in a citrate buffer, pH 6.0 . Samples were incubated with primary antibody (1/3000) for 20 minutes at 25°C. An undiluted HRP-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.

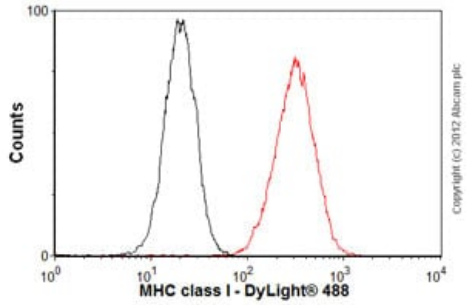
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Immunocytochemistry/ Immunofluorescence - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

ICC/IF image of unpurified **ab52922** stained MCF7 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab52922**, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

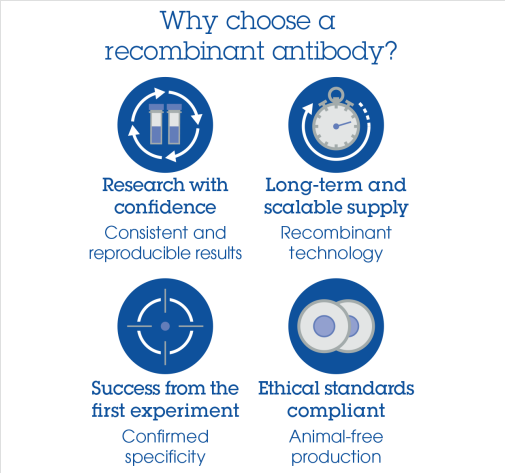
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Flow Cytometry (Intracellular) - Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

Overlay histogram showing Raji cells stained with **ab52922** (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 (**ab52922**, 1/100) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Raji cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52922**).



Anti-HLA A antibody [EP1395Y] - BSA and Azide free (ab216653)

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