Product datasheet

Anti-HLA A antibody [EP1395Y] ab52922

Overview

Product name: Anti-HLA A antibody [EP1395Y]
Description: Rabbit monoclonal [EP1395Y] to HLA A
Host species: Rabbit
Tested applications: Suitable for: IHC-Fr, IHC - Wholemount, In-Cell ELISA, WB, IP, Flow Cyt, IHC-P, ICC/IF
Species reactivity: Reacts with: Rat, Human
Immunogen: Synthetic peptide within Human HLA A aa 50-150. The exact sequence is proprietary.
General notes: 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.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties

Form: Liquid
Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity: Protein A purified
Clonality: Monoclonal
Clone number: EP1395Y
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab52922 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Wholemount</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>In-Cell ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/20. For unpurified use at 1/30.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. For unpurified use at 1/250 - 1/500. See IHC antigen retrieval protocols.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100. For unpurified use at 1/250 - 1/500.</td>
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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.
All lanes: Anti-HLA A antibody [EP1395Y] (ab52922) at 1/10000 dilution

Lane 1: Wild-type A431 whole cell lysate
Lane 2: EPCAM knockout A431 whole cell lysate
Lane 3: A549 whole cell lysate
Lane 4: Jurkat whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

why is the actual band size different from the predicted?

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.
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.

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).
**Western blot - Anti-HLA A antibody [EP1395Y]** (ab52922)

*All lanes:* Anti-HLA A antibody [EP1395Y] (ab52922) at 1/5000 dilution (purified)

*Lane 1:* THP-1 cell lysate at 20 µg

*Lane 2:* A549 cell lysate at 1/20 dilution

**Secondary**

*All lanes:* Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

**Predicted band size:** 41 kDa

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HLA A antibody [EP1395Y] (ab52922)**

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.
Immunoprecipitation - Anti-HLA A antibody [EP1395Y] (ab52922)

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, a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/10,000) was used for detection. A rabbit monoclonal IgG (ab172730) was used instead of ab128913 as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

Flow Cytometry - Anti-HLA A antibody [EP1395Y] (ab52922)

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.
IHC - Wholemount of human zebrafish xenograft labelling HLA A with ab52922. Sample was incubated with primary antibody (1/100) for 1 hours at 4°C. An Alexa Fluor® 647-conjugated goat anti-rabbit IgG polyclonal (1/400) was used as the secondary antibody.

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.

Anti-HLA A antibody [EP1395Y] (ab52922) at 1/2000 dilution (purified) + HL-60 cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 41 kDa

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.
Anti-HLA A antibody [EP1395Y] (ab52922) at 1/10000 dilution + Raji cell lysate at 10 µg

Secondary
Goat anti rabbit IgG HRP conjugated at 1/2000 dilution

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

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.

Ab52922 at 1/250 dilution staining human tonsil; paraffin embedded.
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, a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/10,000) was used for detection. A rabbit monoclonal IgG (ab172730) was used instead of ab128913 as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

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