abcam

Product datasheet

Anti-HLA A antibody [EP1395Y] ab52922

KO 評価済 リコンビナント RabMAb

15 Abreviews 89 References 画像数 15

製品の概要

製品名	Anti-HLA A antibody [EP1395Y]
製品の詳細	Rabbit monoclonal [EP1395Y] to HLA A
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
種交差性	**交差種:** Human
免疫原	Synthetic peptide within Human HLA A aa 50-150. The exact sequence is proprietary. Database link: **P04439**
ポジティブ・コントロール	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.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EP1395Y

アイソタイプ IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab52922の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

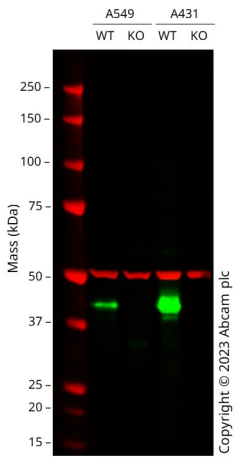
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (4)	1/2000. Predicted molecular weight: 41 kDa. **For unpurified use at 1/10000 - 1/50000.**
IP		1/20. **For unpurified use at 1/30.**
IHC-P	★★★★★ (5)	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**.
ICC/IF	★★☆☆☆ (1)	1/100. **For unpurified use at 1/250 - 1/500.**

ターゲット情報

関連性

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.

画像



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

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

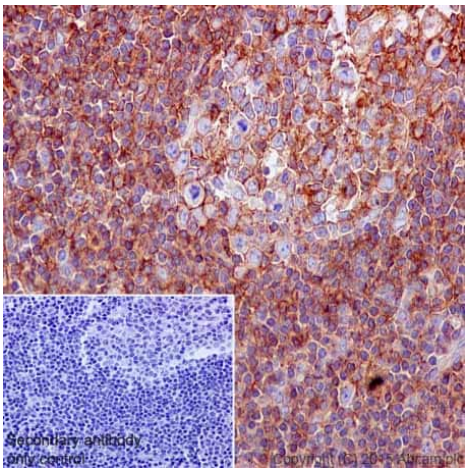
Lane 1 : Wild-type A549 cell lysate
Lane 2 : HLA-A knockout A549 cell lysate
Lane 3 : Wild-type A431 cell lysate
Lane 4 : HLA-A knockout A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

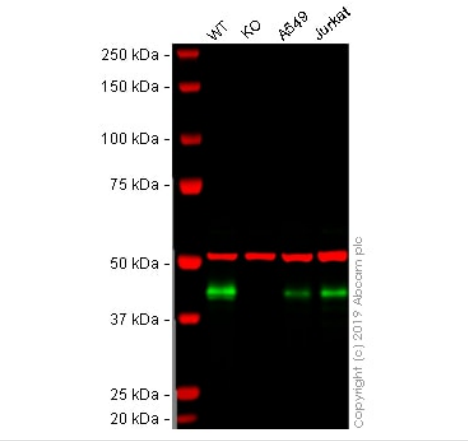
Predicted band size: 41 kDa
Observed band size: 41 kDa

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.



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.



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

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

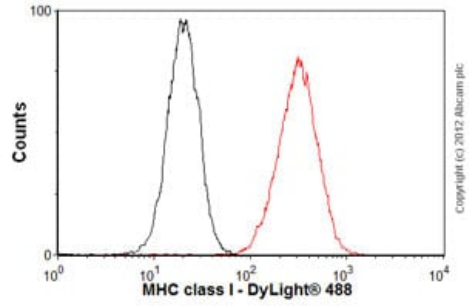
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

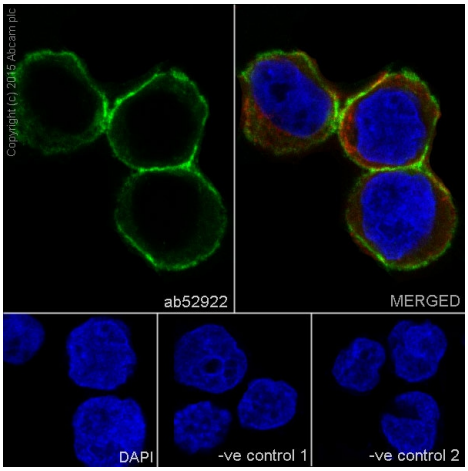
Lanes 1 - 4: Merged signal (red and green). Green - ab52922 observed at 40 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

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.



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

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.

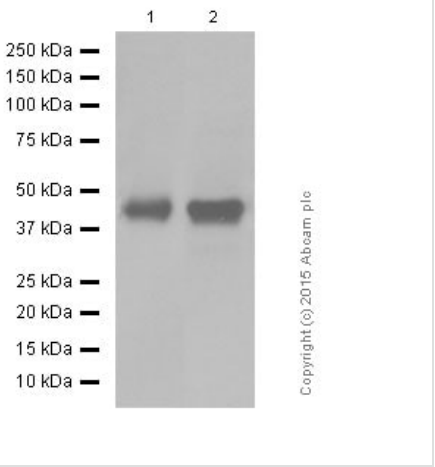


Immunocytochemistry/ Immunofluorescence - Anti-HLA A antibody [EP1395Y] (ab52922)

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)

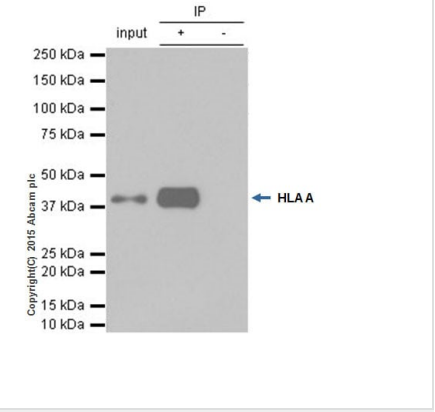
All lanes : Anti-HLA A antibody [EP1395Y] (ab52922) at 1/5000 dilution (purified)

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.

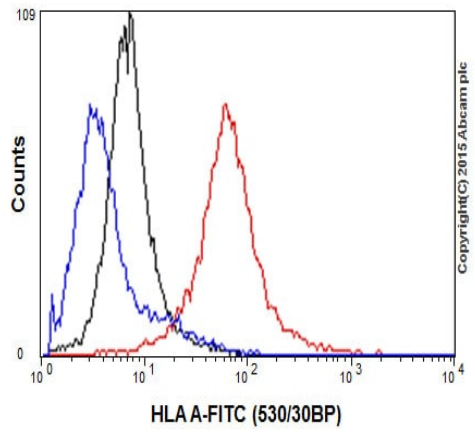


Immunoprecipitation - Anti-HLA A antibody [EP1395Y] (ab52922)

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 intead of **ab128913** as a negative control (Lane 3).

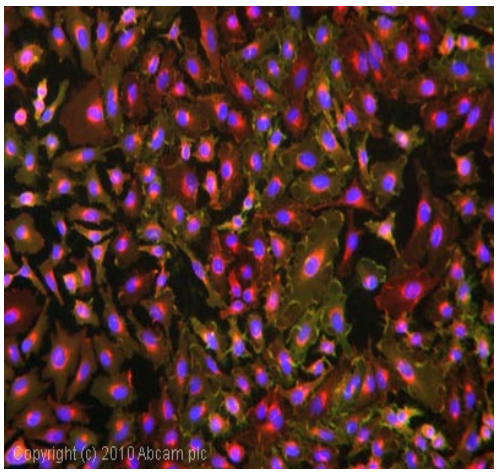
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



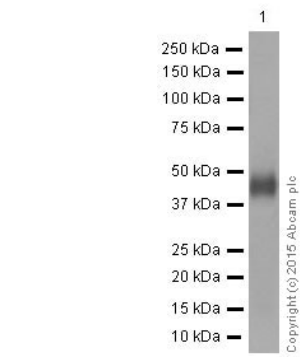
Flow Cytometry (Intracellular) - Anti-HLA A antibody [EP1395Y] (ab52922)

Intracellular Flow Cytometry analysis ofRaji cells labelling HLA Awith 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.



Immunocytochemistry/ Immunofluorescence - Anti-HLA A antibody [EP1395Y] (ab52922)

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.



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

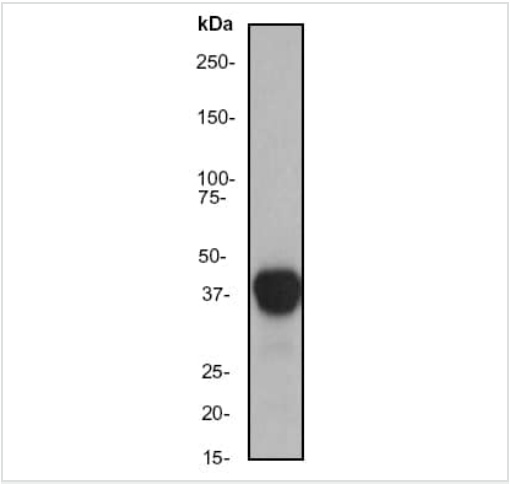
Anti-HLA A antibody [EP1395Y] (ab52922) at 1/2000 dilution (purified) + HL-60 cell lysate at 20 µg

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.



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

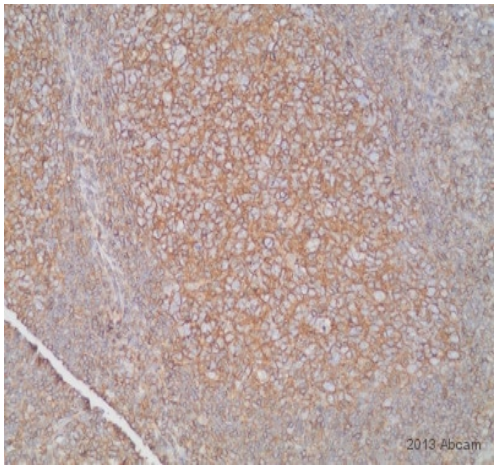
Anti-HLA A antibody [EP1395Y] (ab52922) at 1/10000 dilution + Raji cell lysate at 10 µg

Secondary

Goat anti rabbit IgG HRP conjugated at 1/2000 dilution

Predicted band size: 41 kDa

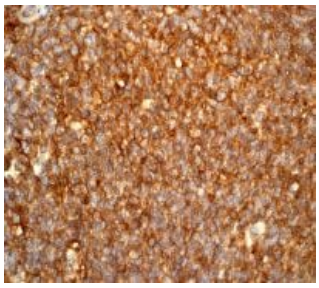
Observed band size: 41 kDa



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

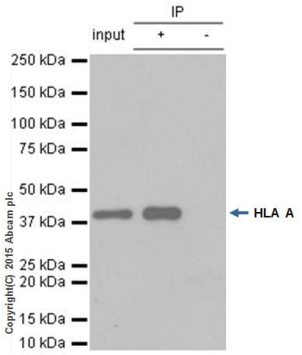
This image is courtesy of an anonymous Abreview

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.



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

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



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

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 intead of **ab128913** as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

Anti-HLA A antibody [EP1395Y] (ab52922)

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