abcam

Product datasheet

Anti-IL3RA/CD123 antibody [EPR24481-20] ab280355

Recombinant RabMAb

9 Images

Overview

Product name	Anti-IL3RA/CD123 antibody [EPR24481-20]
Description	Rabbit monoclonal [EPR24481-20] to IL3RA/CD123
Host species	Rabbit
Tested applications	Suitable for: IP, Flow Cyt, WB, ICC/IF, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human tonsil, HDLM-2, KG-1a lysates. IHC-P: Human tonsil and Hodgkin's lymphoma tissues. ICC/IF: HDLM-2 cells. Flow Cyt: HDLM-2, Human peripheral blood mononuclear cells. IP: HDLM-2 cells
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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24481-20
lsotype	lgG

Applications

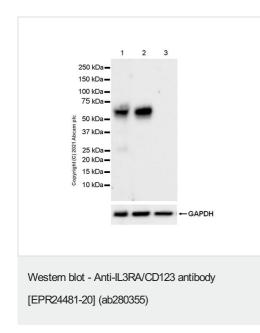
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab280355 in the following tested applications.

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

Application	Abreviews	Notes
IP		1/30.
Flow Cyt		1/500.
WB		1/1000. Predicted molecular weight: 43 kDa.
ICC/IF		1/1000.
IHC-P		1/500.

Target	
Function	This is a receptor for interleukin-3.
Sequence similarities	Belongs to the type I cytokine receptor family. Type 5 subfamily.
Domain	The WSXWS motif appears to be necessary for proper protein folding and thereby efficient intracellular transport and cell-surface receptor binding. The box 1 motif is required for JAK interaction and/or activation.
Cellular localization	Membrane.

Images



All lanes : Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355) at 1/1000 dilution

Lane 1 : Human tonsil tissue lysate at 40 μg Lane 2 : HDLM-2 (human hodgkin lymphoma) whole cell lysate at 20 μg Lane 3 : K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 μg

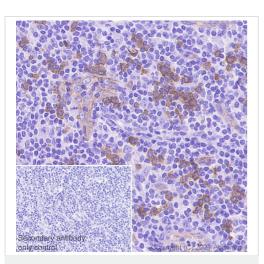
Secondary

Lane 1 : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution Lanes 2-3 : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 43 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST The expression profile/molecular weight observed is consistent with what has been described in the literature (PMID:30647406). **Negative control:** K-562 cell (PMID: 30647406)

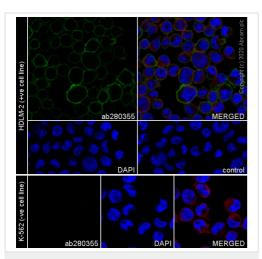
Exposure time: 37 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355) Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling IL3RA/CD123 with ab280355 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human tonsil (PMID: 27340692, PMID:15728234, PMID:28205524). The section was incubated with ab280355 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

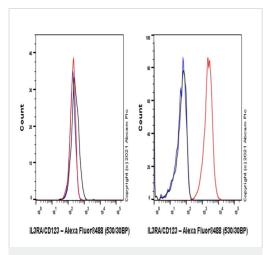


Immunocytochemistry/ Immunofluorescence - Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355) Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HDLM-2 cells labelling IL3RA/CD123 with ab280355 at 1/1000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in HDLM-2 cells.

Negative control: K-562 (PMID: 26531164).

<u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody -Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow cytometric analysis of K-562(Human chronic myelogenous leukemia lymphoblast, Left)/ HDLM-2(Human Hodgkin lymphoma, Right) cells labelling IL3RA/CD123 with ab280355 at 1/500 dilution (0.1ug)/ red compared with a Rabbit monoclonal lgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

Negative control: K-562 cell (PMID: 26531164).

Gated on viable cells.



IF input 250 kDa -150 kDa 🗕 100 kDa 🗕 75 kDa 🗕 50 kDa 🗕 🧕 37 kDa 🗕 25 kDa 🗕 20 kDa 🗕 - IgG light chain 2021 0 15 kDa 🗕 uliuka 10 kDa 🗕 2 1 3

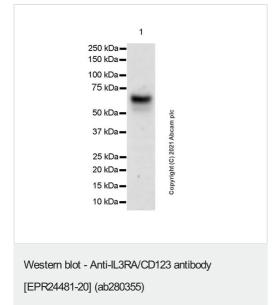
Immunoprecipitation - Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355) IL3RA/CD123 was immunoprecipitated from 0.35 mg HDLM-2 (human hodgkin lymphoma) whole cell lysate 10 µg with ab280355 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab280355 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: HDLM-2 whole cell lysate 10 µg

Lane 2: ab280355 IP in HDLM-2 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab280355 in HDLM-2 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 32 seconds



Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355) at 1/1000 dilution + KG-1a (human bone marrow myeloblast) whole cell lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

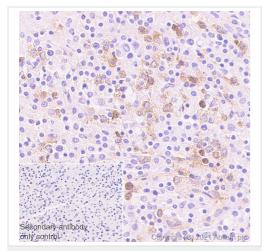
Predicted band size: 43 kDa Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST The expression profile/molecular weight observed is consistent with what has been described in the literature (PMID:30647406). Exposure time: 103 seconds

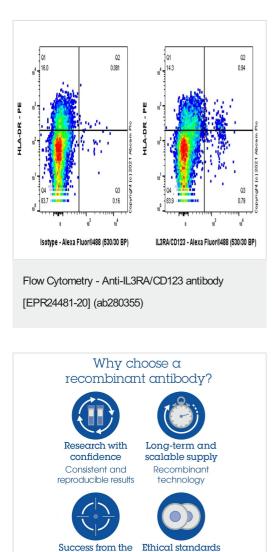
Immunohistochemical analysis of paraffin-embedded human Hodgkin's lymphoma tissue labeling IL3RA/CD123 with ab280355 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human Hodgkin's lymphoma. The section was incubated with ab280355 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IL3RA/CD123 antibody [EPR24481-20] (ab280355)



Flow cytometric analysis of human peripheral blood mononuclear cell (PBMC) cells labelling IL3RA/CD123 with ab280355 at 1/500 dilution (0.1ug)/ Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control. Goat anti rabbit IgG (Alexa Fluor® 488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

Cells were stained with rabbit IgG or ab280355. Then stained with anti-HLA-DR conjugated to PE.

Gated on viable cells.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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first experiment

Confirmed

specificity

(ab280355)

Anti-IL3RA/CD123 antibody [EPR24481-20]

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