abcam

Product datasheet

Anti-CD81 antibody [TS81] - BSA and Azide free ab59477

KO VALIDATED

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Overview

Product name	Anti-CD81 antibody [TS81] - BSA and Azide free
Description	Mouse monoclonal [TS81] to CD81 - BSA and Azide free
Host species	Mouse
Specificity	Recognises the TAPA-1 antigen, a 23 kDa (smear) protein,
Tested applications	**Suitable for:** IHC-P, Flow Cyt, WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to CD81. Jurkat cell line
Positive control	This antibody gave a positive result in IHC in the following FFPE tissue: Human normal lung. Flow Cyt: HAP1-WT cells. WB: Raji (Human Burkitt's lymphoma cell line) whole cell lysate
General notes	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. 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

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	pH: 7.30 Constituent: 100% PBS Sterile-filtered through 0.22 µm. Carrier and preservative free
Carrier free	Yes

Purity	Ion Exchange Chromatography
Purification notes	ab59477 is sterile-filtered through 0.22 µm and treated to remove endotoxins.
Clonality	Monoclonal
Clone number	TS81
Myeloma	x63-Ag8.653
Isotype	IgG2a
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab59477 in the following tested applications.

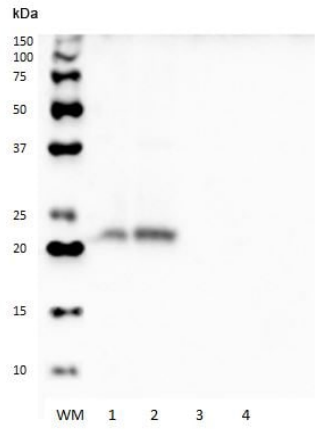
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		Use 0.5µg for 10^6 cells. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Use under non reducing condition. Predicted molecular weight: 25 kDa.
ICC/IF		Use a concentration of 1 µg/ml.

Target

Function	May play an important role in the regulation of lymphoma cell growth. Interacts with a 16-kDa Leu-13 protein to form a complex possibly involved in signal transduction. May acts a the viral receptor for HCV.
Tissue specificity	Hematolymphoid, neuroectodermal and mesenchymal tumor cell lines.
Involvement in disease	Defects in CD81 are the cause of immunodeficiency common variable type 6 (CVID6) [MIM:613496]; also called antibody deficiency due to CD81 defect. CVID6 is a primary immunodeficiency characterized by antibody deficiency, hypogammaglobulinemia, recurrent bacterial infections and an inability to mount an antibody response to antigen. The defect results from a failure of B-cell differentiation and impaired secretion of immunoglobulins; the numbers of circulating B cells is usually in the normal range, but can be low.
Sequence similarities	Belongs to the tetraspanin (TM4SF) family.
Post-translational modifications	Not glycosylated.
Cellular localization	Membrane.

Images



Western blot - Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

All lanes : Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

Lane 1 : Positive cell line Raji (Human Burkitt's lymphoma cell line) whole cell lysate at 25 µg
Lane 2 : Positive cell line total protein in non-reducing conditions at 50 µg
Lane 3 : Negative cell line U266 at 25 µg
Lane 4 : Negative cell line total protein in non-reducing conditions at 50 µg

Predicted band size: 25 kDa

Western Blot under non-reducing conditions

Detection: chemiluminescence

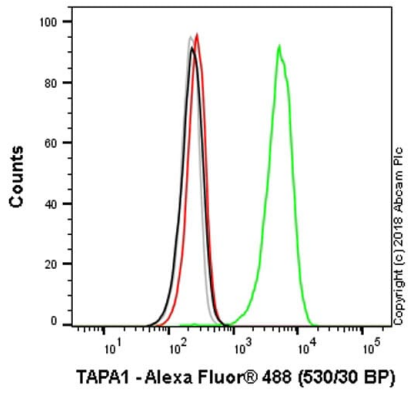
Immunocytochemistry/ Immunofluorescence - Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

ICC/IF image of CD81 stained Hek293 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 (ab59477, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse 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.

Flow Cytometry - Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

Overlay histogram showing Jurkat cells stained with ab59477 (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 (ab59477, 0.5µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 1µg/1x10^6 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.



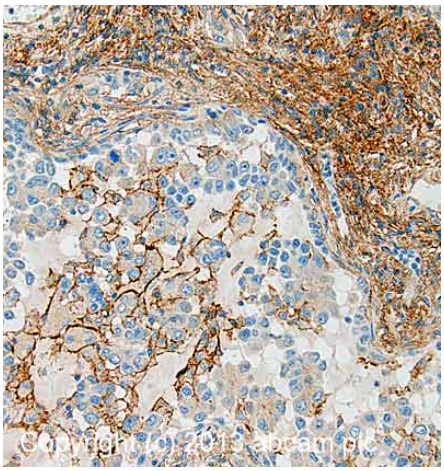
Flow Cytometry - Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CD81 knockout cells (red line) stained with ab59477. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab59477, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) presorbed (**ab150117**) at 1/2000 dilution for 30 min at 22°C.

A mouse IgG1 isotype control antibody (**ab170190**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CD81 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min), , permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD81 antibody [TS81] - BSA and Azide free (ab59477)

IHC image of CD81 staining in Human normal lung formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab59477, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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