abcam

Product datasheet

Anti-TAP2 antibody [EPR26237-82] ab307282

KO VALIDATED

Recombinant RabMAb

12 Images

Overview

Product name	Anti-TAP2 antibody [EPR26237-82]	
Description	Rabbit monoclonal [EPR26237-82] to TAP2	
Host species	Rabbit	
Specificity	Not suitable for rat and mouse for WB and IHC-P.	
Tested applications	Suitable for: IHC-P, ICC/IF, IP, WB, Flow Cyt (Intra)	
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat	
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Wild-type HeLa whole cell lysate. Jurkat, Raji, MOLT-4 and HT-1080 whole cell lysate. Untreated HeLa whole cell lysate. IFN gamma treated HeLa whole cell lysate. Human tonsil tissue lysate. IHC-P: Human tonsil, kidney, liver, breast and cerebrum tissue. Human breast cancer tissue. Wild -type HeLa cell pellet. ICC/IF: Wild-type HeLa cells. Flow Cyt (Intra): Wild-type HeLa cells. IP: IFN gamma treated HeLa whole cell lysate.	
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. 	

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. Avoid freeze / thaw cycle. Storage buffer pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR26237-82
lsotype	lgG

Applications

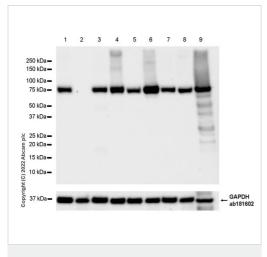
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab307282 in the following tested applications.

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

Application	Abreviews	Notes
ІНС-Р		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/30.
WB		1/1000. Predicted molecular weight: 76 kDa.
Flow Cyt (Intra)		1/500.

Target

Function	Involved in the transport of antigens from the cytoplasm to the endoplasmic reticulum for association with MHC class I molecules. Also acts as a molecular scaffold for the final stage of MHC class I folding, namely the binding of peptide. Nascent MHC class I molecules associate with TAP via tapasin. Inhibited by the covalent attachment of herpes simplex virus ICP47 protein, which blocks the peptide-binding site of TAP. Inhibited by human cytomegalovirus US6 glycoprotein, which binds to the lumenal side of the TAP complex and inhibits peptide translocation by specifically blocking ATP-binding to TAP1 and prevents the conformational rearrangement of TAP induced by peptide binding. Inhibited by human adenovirus E3-19K glycoprotein, which binds the TAP complex and acts as a tapasin inhibitor, preventing MHC class VTAP association.
Involvement in disease	Bare lymphocyte syndrome 1
Sequence similarities	Belongs to the ABC transporter superfamily. ABCB family. MHC peptide exporter (TC 3.A.1.209) subfamily. Contains 1 ABC transmembrane type-1 domain. Contains 1 ABC transporter domain.
Domain	The peptide-binding site is shared between the cytoplasmic loops of TAP1 and TAP2.
Cellular localization	Endoplasmic reticulum membrane. The transmembrane segments seem to form a pore in the membrane.



Western blot - Anti-TAP2 antibody [EPR26237-82] (ab307282)

All lanes : Anti-TAP2 antibody [EPR26237-82] (ab307282) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : TAP2 knockout HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3 : Jurkat (human T cell leukemia T lymphocyte from peripheral blood) whole cell lysate

Lane 4 : Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lane 5 : Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 6 : HeLa treated with 10 ng/ml IFN gamma for 16 hours whole cell lysate

Lane 7 : MOLT-4 (human lymphoblastic leukemia T lymphoblast) whole cell lysate

Lane 8 : HT-1080 (human fibrosarcoma epithelial cell) whole cell lysate

Lane 9 : Human tonsil tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 76 kDa Observed band size: 75 kDa

Samples are non-boiled as boiling may cause protein aggregates.

The expression of TAP2 is upregulated in response to IFN gamma treatment (PMID: 1946428).

Blocking/dilution buffer: 5% NFDM/TBST.

Exposure time: 26 seconds.

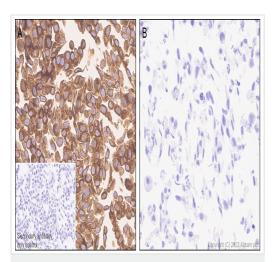


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human tonsil. The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

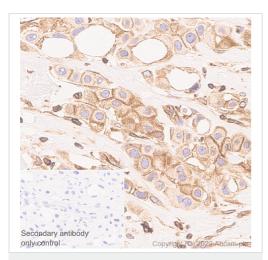
Immunohistochemical analysis of paraffin-embedded cell pellets labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Positive staining in wild-type HeLa cell pellet and no staining in TAP2 knockout HeLa cell pellet.

The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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

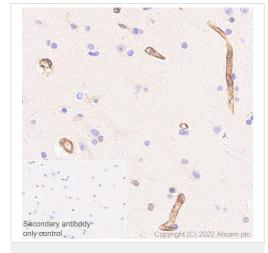


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human breast cancer (PMID: 22065046). The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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

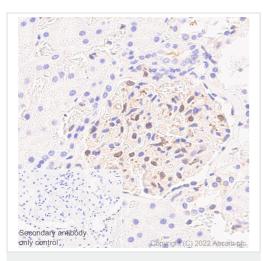


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human cerebrum. The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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

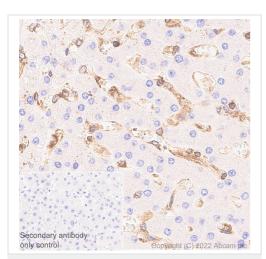


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human kidney. The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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

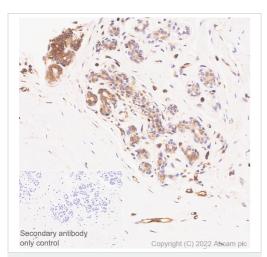


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human liver. The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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

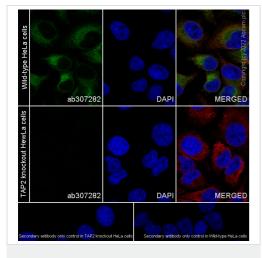


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TAP2 antibody [EPR26237-82] (ab307282)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling TAP2 with AB307282 at 1/2000 (0.257 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining in human breast. The section was incubated with ab307282 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 ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

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



Immunocytochemistry/ Immunofluorescence - Anti-TAP2 antibody [EPR26237-82] (ab307282)

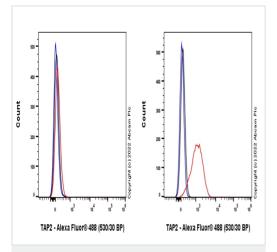
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized TAP2 KO HeLa (TAP2 knockout human cervical adenocarcinoma epithelial cell) (<u>ab265426</u>) cells labeling TAP2 with AB307282 at 1/500 (1.028 ug/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green).

Confocal image showing endoplasmic reticulum staining in wildtype HeLa cells and no staining in TAP2 knockout HeLa cells.

Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

<u>ab12223</u> Anti-KDEL mouse monoclonal antibody was used to counterstain tubulin at 1/200 dilution (5 ug/ml) dilution, followed by <u>ab150120</u> Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at a 1/1000 dilution (2 ug/ml) (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Wild-type HeLa (human cervix adenocarcinoma epithelial cell, Right) / TAP2 knockout HeLa (Left) cells labeling TAP2 with AB307282 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, <u>ab150081</u>) at 1/2000 dilution was used as the secondary antibody.

Positive staining on HeLa cells (<u>ab255928</u>), while no staining on TAP2 knockout HeLa cells (<u>ab265426</u>).

Flow Cytometry (Intracellular) - Anti-TAP2 antibody [EPR26237-82] (ab307282)



Immunoprecipitation - Anti-TAP2 antibody [EPR26237-82] (ab307282) TAP2 was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) treated with 10 ng/ml IFN gamma for 16 hours, whole cell lysate 10 ug with AB307282 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using AB307282 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa treated with 10 ng/ml IFN gamma for 16 hours, whole cell lysate 10 ug

Lane 2: AB307282 IP in HeLa treated with 10 ng/ml IFN gamma for 16 hours, whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab307282 in HeLa treated with 10ng/ml IFN gamma for 16 hours, whole cell lysate

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

Exposure time: 1 seconds.

Input is non-boiled as boiling may cause protein aggregates.



Anti-TAP2 antibody [EPR26237-82] (ab307282)

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

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