# abcam

### Product datasheet

## Anti-CD80 antibody [EPR1157(2)] ab134120

KO VALIDATED Recombinant RabMAb

#### Overview

Product name	Anti-CD80 antibody [EPR1157(2)]
Description	Rabbit monoclonal [EPR1157(2)] to CD80
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P Unsuitable for: ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji and Ramos cell lysates. IHC-P: Human tonsil tissue. Flow Cyt (intra): Raji cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>
	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

#### **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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR1157(2)
lsotype	lgG

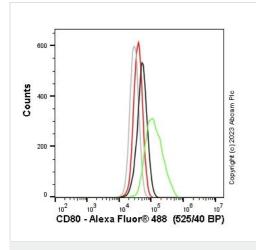
#### Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab134120 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 60 kDa (predicted molecular weight: 33 kDa). <b>For unpurified use at 1/500.</b>
IHC-P	★★★★	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Application notes	Is unsuitable for ICC/IF.	
Target		
Function	•	signal essential for T-lymphocyte activation. T-cell proliferation and d by the binding of CD28 or CTLA-4 to this receptor.
Tissue specificity	Expressed on activated B-ce	lls, macrophages and dendritic cells.
Sequence similarities	Contains 1 lg-like C2-type (in Contains 1 lg-like V-type (imr	
Cellular localization	Membrane.	

Images



Flow Cytometry (Intracellular) - Anti-CD80 antibody [EPR1157(2)] (ab134120)

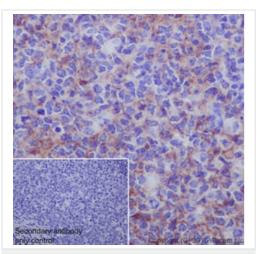
Flow cytometry overlay histogram showing wild-type Raji (green line) and CD80 knockout Raji stained with ab134120 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10 $\mu$ g/ml human lgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab134120) (1x 10<sup>6</sup> in 100 $\mu$ l at 0.2  $\mu$ g/ml (1/10150)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Raji - black line, CD80 knockout Raji - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

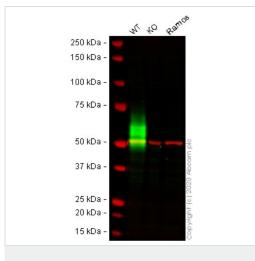
This antibody gave a positive signal in Raji Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD80 antibody [EPR1157(2)] (ab134120)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD80 with ab134120 at a dilution of 1/1000. Heat mediated antigen retrieval was performed using Trs/EDTA buffer, pH9 (<u>ab93684</u>). ImmunoHistoProbe one step HRP Polymer was used. Counter stained with hematoxylin.

The image shows cytoplasmic and membrane staining.



Western blot - Anti-CD80 antibody [EPR1157(2)] (ab134120)

All lanes : Anti-CD80 antibody [EPR1157(2)] (ab134120) at 1/1000 dilution

Lane 1 : Wild-type Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 2 : CD80 knockout Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 3 : Ramos (Human Burkitt's lymphoma cell line) whole cell lysate

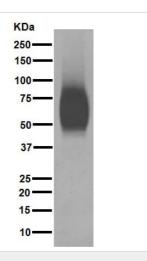
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa Observed band size: 55 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab134120 observed at 55 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab134120 was shown to react with CD80 in wild-type Raji cells in western blot with loss of signal observed in CD80 knockout sample. Wild-type and CD80 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before incubation with ab134120 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CD80 antibody [EPR1157(2)] (ab134120)

Western blot - Anti-CD80 antibody [EPR1157(2)] (ab134120) Anti-CD80 antibody [EPR1157(2)] (ab134120) at 1/2500 dilution (purified) + Raji cell lysate at 20 µg

#### Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 33 kDa Observed band size: 60 kDa

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

**All lanes :** Anti-CD80 antibody [EPR1157(2)] (ab134120) at 1/1000 dilution (unpurified)

Lane 1 : Raji cell lysate Lane 2 : Ramos cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

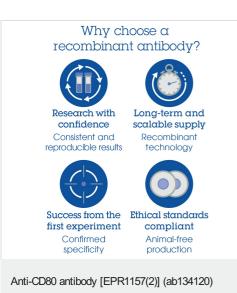
**All lanes :** Goat anti-rabbit HRP conjugated antibody at 1/2000 dilution

Predicted band size: 33 kDa Observed band size: 60 kDa

			and menounal (m	A) data for ab134120			
Normal fissue samples			Malignant tissue samples				
Human cardiac muscle	x	Human placenta	x	Clear cell carcinoma of human kidney	x	Human lung carcinoma	x
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x	Human non- Hodgkin's lymphoma	1
Human colon	¥ (immune cells √)	Human skin	× (immune cells 🗸 )	Human breast carcinoma	x	Human ovarian carcinoma	x
Human endometrium	x	Human spleen	×	Human cervical carcinoma	×	Human poncreatic carcinoma	x
Human kidney	x	Human stornach	×	Human colon carcinoma	x	Human prostatic hyperplasia	×
Human liver	x	Human testis	×	Human endometrial carcinoma	x	Human Ihymoma	x
Human lung	x	Human thyroid	x	Human gastric carcinoma	x	Human thymus hyperplasia	x
Human mammary gland	x	Human tonsi	1	Human glioma	x	Human thyroid carcinoma	x
Human pancreas	x			Human hepatocellular carcinoma	x		

Tissue Microarrays stained for "Anti-CD80 antibody [EPR1157(2)]" using "ab134120" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond<sup>™</sup> Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab134120 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD80 antibody [EPR1157(2)] (ab134120)



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