# abcam

# Product datasheet

# Anti-CD22 antibody [EPR20061] ab207727



★★★★ 2 Abreviews 10 Images

#### Overview

Product name Anti-CD22 antibody [EPR20061]

**Description** Rabbit monoclonal [EPR20061] to CD22

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP

Species reactivity Reacts with: Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Ramos, Daudi and Raji whole cell lysates; Human tonsil and fetal spleen lysates. IHC-P:

Human spleen and tonsil tissues. ICC/IF: Daudi and Raji cells. IP: Human tonsil lysate; Raji 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **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 long

term. Avoid freeze / thaw cycle.

**Storage buffer** pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR20061

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**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab207727 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
WB		1/1000. Detects a band of approximately 130 kDa (predicted molecular weight: 95 kDa).
IHC-P	* * * * * <u>(2)</u>	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/30.

#### **Target**

**Function** 

Mediates B-cell B-cell interactions. May be involved in the localization of B-cells in lymphoid tissues. Binds sialylated glycoproteins; one of which is CD45. Preferentially binds to alpha-2,6-linked sialic acid. The sialic acid recognition site can be masked by cis interactions with sialic acids on the same cell surface. Upon ligand induced tyrosine phosphorylation in the immune response seems to be involved in regulation of B-cell antigen receptor signaling. Plays a role in positive regulation through interaction with Src family tyrosine kinases and may also act as an inhibitory receptor by recruiting cytoplasmic phosphatases via their SH2 domains that block signal transduction through dephosphorylation of signaling molecules.

Tissue specificity

B-lymphocytes.

Sequence similarities

Belongs to the immunoglobulin superfamily. SIGLEC (sialic acid binding lg-like lectin) family. Contains 6 lg-like C2-type (immunoglobulin-like) domains.

Contains 1 lg-like V-type (immunoglobulin-like) domain.

Domain

Contains 4 copies of a cytoplasmic motif that is referred to as the immunoreceptor tyrosine-based

inhibitor motif (ITIM). This motif is involved in modulation of cellular responses. The

phosphorylated ITIM motif can bind the SH2 domain of several SH2-containing phosphatases.

Post-translational modifications

 $Phosphorylation of Tyr-762, Tyr-807 \ and \ Tyr-822 \ are involved in binding to \ SYK, GRB2 \ and \$ 

respectively. Phosphorylation of Tyr-842 is involved in binding to SYK, PLCG2 and

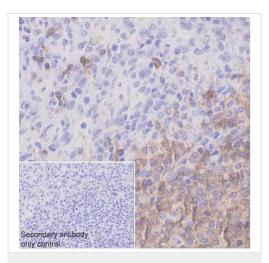
PIK3R1/PIK3R2.

Phosphorylated on tyrosine residues by LYN.

**Cellular localization** 

Cell membrane.

#### **Images**

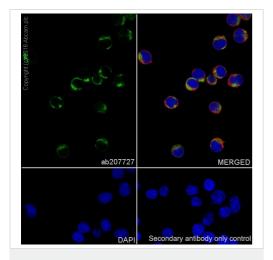


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD22 antibody
[EPR20061] (ab207727)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling CD22 with ab207727 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membranous and cytoplasmic staining on B cells of human spleen [PMID: 11967115]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

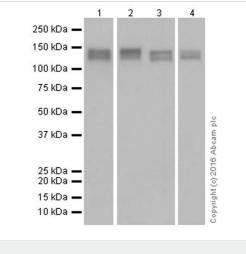


Immunocytochemistry/ Immunofluorescence - Anti-CD22 antibody [EPR20061] (ab207727)

Immunofluorescent analysis of 100% methanol-fixed Daudi (Human Burkitt's lymphoma cell line) cells labeling CD22 with ab207727 at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Daudi cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with <a href="mailto:ab195889">ab195889</a> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) at 1/1000 dilution.



Western blot - Anti-CD22 antibody [EPR20061] (ab207727)

**Lanes 1 & 4 :** Anti-CD22 antibody [EPR20061] (ab207727) at 1/2000 dilution

**Lanes 2-3**: Anti-CD22 antibody [EPR20061] (ab207727) at 1/10000 dilution

**Lane 1 :** Ramos (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 2 : Daudi (Human Burkitt's lymphoma cell line) whole cell lysate

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

Lane 4: Human tonsil lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

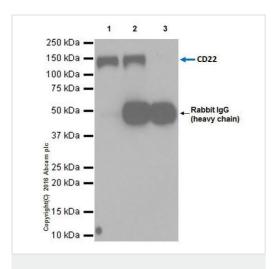
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

**Predicted band size:** 95 kDa **Observed band size:** 130 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 3 seconds; Lane 2/3: 1 second; Lane 4: 8 seconds.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 24264377 & 21178016).



Immunoprecipitation - Anti-CD22 antibody [EPR20061] (ab207727) CD22 was immunoprecipitated from 0.35 mg of Human tonsil lysate with ab207727 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab207727 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

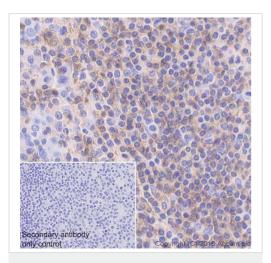
Lane 1: Human tonsil lysate, 10 µg (Input).

Lane 2: ab207727 IP in Human tonsil lysate.

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of ab207727 in Human tonsil lysate.

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

Exposure time: 10 seconds.

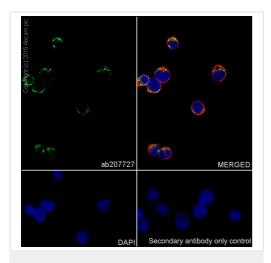


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD22 antibody
[EPR20061] (ab207727)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD22 with ab207727 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membranous and cytoplasmic staining on B cells of human tonsil [PMID: 11967115]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

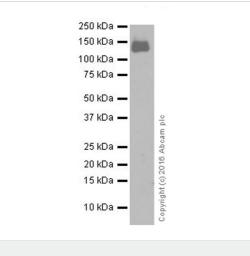


Immunocytochemistry/ Immunofluorescence - Anti-CD22 antibody [EPR20061] (ab207727)

Immunofluorescent analysis of 100% methanol-fixed Raji (Human Burkitt's lymphoma cell line) cells labeling CD22 with ab207727 at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Raji cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with <a href="mailto:ab195889">ab195889</a> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



Western blot - Anti-CD22 antibody [EPR20061] (ab207727)

Anti-CD22 antibody [EPR20061] (ab207727) at 1/1000 dilution + Human fetal spleen lysate at 10  $\mu g$ 

# **Secondary**

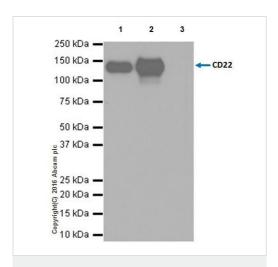
VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

**Predicted band size:** 95 kDa **Observed band size:** 130 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 24264377 & 21178016).



Immunoprecipitation - Anti-CD22 antibody [EPR20061] (ab207727)

CD22 was immunoprecipitated from 0.35 mg of Raji (Human Burkitt's lymphoma cell line) whole cell lysate with ab207727 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab207727 at 1/2000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

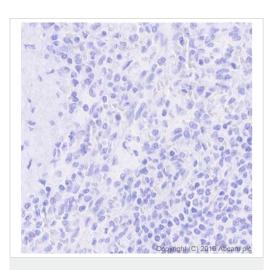
Lane 1: Raji whole cell lysate, 10 µg (Input).

Lane 2: ab207727 IP in Raji whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of ab207727 in Raji whole cell lysate.

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

Exposure time: 10 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD22 antibody
[EPR20061] (ab207727)

Immunohistochemical analysis of paraffin-embedded human thymoma tissue labeling CD22 with ab207727 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

**Negative control:** no staining on human thymoma [PMID: 11967115].

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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