abcam

Product datasheet

Anti-CD33 antibody [EPR24370-124] - BSA and Azide free ab281568





RabMAb

*** * 2 Abreviews 9 Images

Overview

Product name Anti-CD33 antibody [EPR24370-124] - BSA and Azide free

Description Rabbit monoclonal [EPR24370-124] to CD33 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Human

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

Positive control WB: THP-1, HL-60, TF-1 lysates. IHC-P: Human colon, liver, tonsil, Hodgkin's lymphoma and clear

renal carcinoma tissues.

General notes Please Note: Clone [EPR24370-124] (this product) is different to that of ab199432 [SP266] for

the same target.

ab281568 is the carrier-free version of ab270942.

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

Storage buffer Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number EPR24370-124

Isotype IgG

Applications

The Abpromise guarantee O

Our **Abpromise guarantee** covers the use of ab281568 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	★★★★★ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 40 kDa.

Application notes

Is unsuitable for Flow Cyt,ICC/IF or IP.

Target

Function

Putative adhesion molecule of myelomonocytic-derived cells that mediates sialic-acid dependent binding to cells. Preferentially binds to alpha-2,6-linked sialic acid. The sialic acid recognition site may be masked by cis interactions with sialic acids on the same cell surface. In the immune response, may act as an inhibitory receptor upon ligand induced tyrosine phosphorylation by recruiting cytoplasmic phosphatase(s) via their SH2 domain(s) that block signal transduction through dephosphorylation of signaling molecules. Induces apoptosis in acute myeloid leukemia (in vitro).

Tissue specificity Monoc

Monocytic/myeloid lineage cells.

Sequence similarities Belongs to the immunoglobulin superfamily. SIGLEC (sialic acid binding lg-like lectin) family.

Contains 1 lg-like C2-type (immunoglobulin-like) domain. Contains 1 lg-like V-type (immunoglobulin-like) domain.

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DomainContains 2 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.

Phosphorylation of Tyr-340 is involved in binding to PTPN6 and PTPN11. Phosphorylation of Tyr-

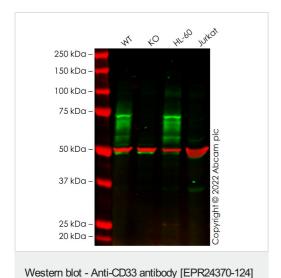
358 is involved in binding to PTPN6.

Cell membrane.

Post-translational modifications

Cellular localization

Images



All lanes : Anti-CD33 antibody [EPR24370-124] (**ab270942**) at 1/1000 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: CD33 knockout THP-1 cell lysate

Lane 3 : HL-60 cell lysate

Lane 4 : Jurkat cell lysate

Lysates/proteins at 10 µg per lane.

Performed under reducing conditions.

Predicted band size: 40 kDa

Observed band size: 60-80 kDa

- BSA and Azide free (ab281568)

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

False colour image of Western blot: Anti-CD33 antibody [EPR24370-124] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab270942 was shown to bind specifically to CD33. A band was observed at 60-80 kDa in wild-type THP-1 cell lysates with no signal observed at this size in CD33 knockout cell line ab273831 (knockout cell lysate ab273785). To generate this image, wild-type and CD33 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000

1 2 3 4

250 kDa = 150 kDa = 100 kDa = 75 kDa = 20 kDa = 20 kDa = 20 kDa = 100 kDa = 4 GAPDH

Western blot - Anti-CD33 antibody [EPR24370-124] - BSA and Azide free (ab281568)

dilution.

All lanes : Anti-CD33 antibody [EPR24370-124] (**ab270942**) at 1/1000 dilution

Lane 1 : THP-1 (human monocytic leukemia monocyte) whole cell lysate

Lane 2 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 3: HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate

Lane 4 : TF-1 (human erythroleukemia erythroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 40 kDa **Observed band size:** 67-75 kDa

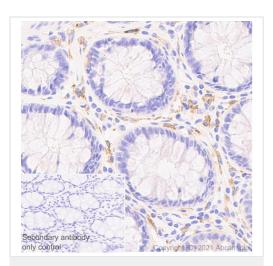
This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

The expression molecular weight observed is consistent with what has been described in the literature (PMID:16380601, 30519686).

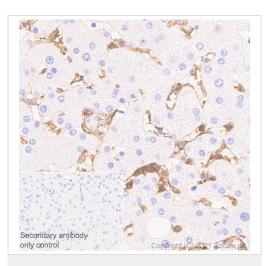
Negative Control: Jurkat (PMID:30519686).

Exposure time: Lanes 1-3:136 seconds; Lane 4:26 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD33 antibody

[EPR24370-124] - BSA and Azide free (ab281568)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD33 antibody
[EPR24370-124] - BSA and Azide free (ab281568)

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human colon tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous and cytoplasmic staining on immune cells of human colon (PMID: 31462392). The section was incubated with <u>ab270942</u> 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

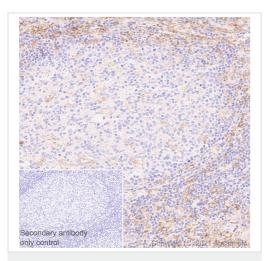
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human liver tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous and cytoplasmic staining on Kupffer cells of human liver (PMID:25721896). The section was incubated with <u>ab270942</u> 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 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-CD33 antibody

[EPR24370-124] - BSA and Azide free (ab281568)

Secondary antibody only control.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD33 antibody
[EPR24370-124] - BSA and Azide free (ab281568)

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous and cytoplasmic staining in human tonsil. The section was incubated with <u>ab270942</u> 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

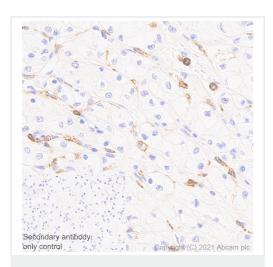
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

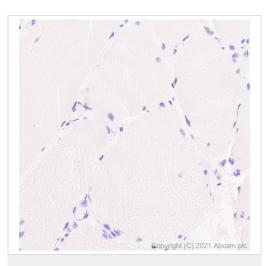
Immunohistochemical analysis of paraffin-embedded Human Hodgkin's lymphoma tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous and cytoplasmic staining in human Hodgkin's lymphoma. The section was incubated with <u>ab270942</u> 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 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-CD33 antibody
[EPR24370-124] - BSA and Azide free (ab281568)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD33 antibody
[EPR24370-124] - BSA and Azide free (ab281568)

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human clear cell renal cell carcinoma tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Membranous and cytoplasmic staining on scattered cells of human clear cell renal cell carcinoma. The section was incubated with <u>ab270942</u> 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

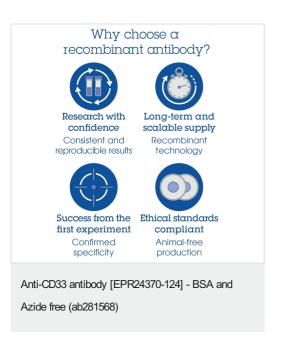
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab270942</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labelling CD33 with <u>ab270942</u> at 1/1000 dilution (0.472 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). **Negative control**: No staining in human skeletal muscle. The section was incubated with <u>ab270942</u> 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

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



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