# abcam

# Product datasheet

# Anti-ICOS antibody [EPR20560] ab224644



★★★★ 1 Abreviews 3 References 10 Images

#### Overview

**Product name** Anti-ICOS antibody [EPR20560]

Rabbit monoclonal [EPR20560] to ICOS **Description** 

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, IP

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: His-tagged human ICOS (aa21-140-GGGGS-aa162-199) recombinant protein; human

thymus and tonsil tissue lysate; HuT-78 cell lysate. IP: Human tonsil tissue lysate. IHC-P: Human

tonsil, stomach and cervix cancer tissue; mouse spleen tissue; rat spleen tissue.

**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

For more information see here.

- Animal-free production

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR20560

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab224644 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 27-29 kDa (predicted molecular weight: 22 kDa).
IHC-P	<b>★</b> ★ ★ ★ ★ (1)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

#### **Target**

#### **Function**

Enhances all basic T-cell responses to a foreign antigen, namely proliferation, secretion of lymphokines, up-regulation of molecules that mediate cell-cell interaction, and effective help for antibody secretion by B-cells. Essential both for efficient interaction between T and B-cells and for normal antibody responses to T-cell dependent antigens. Does not up-regulate the production of interleukin-2, but superinduces the synthesis of interleukin-10. Prevents the apoptosis of preactivated T-cells. Plays a critical role in CD40-mediated class switching of immunoglobin isotypes.

## **Tissue specificity**

Activated T-cells. Highly expressed on tonsillar T-cells, which are closely associated with B-cells in the apical light zone of germinal centers, the site of terminal B-cell maturation. Expressed at lower levels in thymus, lung, lymph node and peripheral blood leukocytes. Expressed in the medulla of fetal and newborn thymus.

### Involvement in disease

Immunodeficiency, common variable, 1

# Sequence similarities

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

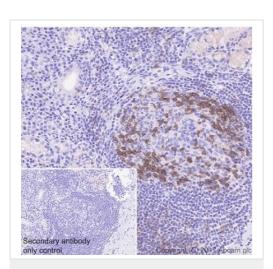
# Post-translational modifications

N-glycosylated.

# Cellular localization

Secreted and Cell membrane.

### **Images**

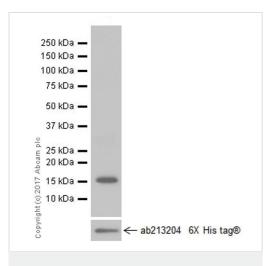


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICOS antibody
[EPR20560] (ab224644)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling ICOS with ab224644 at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic and membranous staining in a population of lymphoid cells of human stomach (magnification x200) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-ICOS antibody [EPR20560] (ab224644)

Anti-ICOS antibody [EPR20560] (ab224644) at 1/1000 dilution + His-tagged human ICOS (aa21-140-GGGGS-aa162-199) recombinant protein, 10 ng

#### **Secondary**

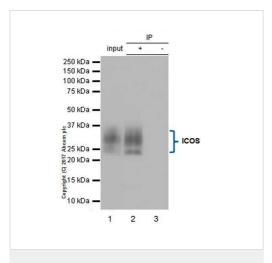
Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit IgG H&L (HRP))

Developed using the ECL technique.

Predicted band size: 22 kDa Observed band size: 17 kDa

Exposure time: 1 second

Blocking and dilution buffer: 5% NFDM/TBST



Immunoprecipitation - Anti-ICOS antibody [EPR20560] (ab224644)

ICOS was immunoprecipitated from 0.35 mg of human tonsil tissue lysate with ab224644 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab224644 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

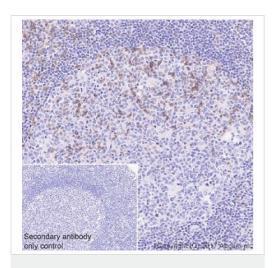
Lane 1: Human tonsil tissue lysate 10 µg (Input).

Lane 2: ab224644 IP in human tonsil tissue lysate (+).

Lane 3: Rabbit monoclonal  $\lg G (\underline{ab172730})$  instead of ab224644 in human tonsil tissue lysate (-).

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

Exposure time: 1 second.

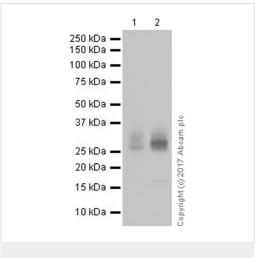


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICOS antibody
[EPR20560] (ab224644)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling ICOS with ab224644 at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic and membranous staining in a population of lymphoid cells of human tonsil (PMID: 20207847), (magnification x200) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-ICOS antibody [EPR20560] (ab224644)

**All lanes :** Anti-ICOS antibody [EPR20560] (ab224644) at 1/1000 dilution

Lane 1: Human tymus tissue lysate

Lane 2 : HuT-78 (human Sezary syndrome cutaneous T

lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG H&L (HRP))

Developed using the ECL technique.

Predicted band size: 22 kDa

Observed band size: 27-29 kDa

Exposure time: 10 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 11169414).

1 2

250 kDa —
150 kDa —
150 kDa —
75 kDa —
25 kDa —
25 kDa —
20 kDa —
15 kDa —
15 kDa —
15 kDa —
10 kDa —

Western blot - Anti-ICOS antibody [EPR20560] (ab224644)

**All lanes :** Anti-ICOS antibody [EPR20560] (ab224644) at 1/1000 dilution

Lane 1: Untreated human tonsil tissue lysate

Lane 2: Human tonsil tissue lysate treated with PNGase F

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG H&L (HRP))

Developed using the ECL technique.

Predicted band size: 22 kDa

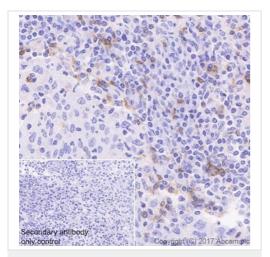
Observed band size: 22,27-29 kDa

Exposure time: 10 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

When untreated two distinct bands of 27kDa and 29kDa were observed. Treatment with PNGase F resulted in a single band with a molecular of 22kDa.

The expression profile observed is consistent with what has been described in the literature (PMID: 11169414).

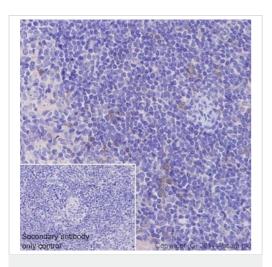


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICOS antibody
[EPR20560] (ab224644)

Immunohistochemical analysis of paraffin-embedded human cervix cancer tissue labeling ICOS with ab224644 at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic and membranous staining in tumor infiltrating lymphoid cells of human cervix cancer (PMID: 23802069) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

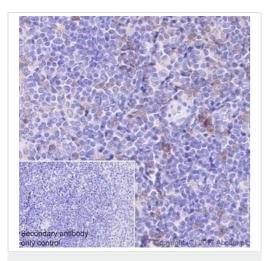


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICOS antibody
[EPR20560] (ab224644)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling ICOS with ab224644 at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic and membranous staining in a population of lymphoid cells of mouse spleen is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

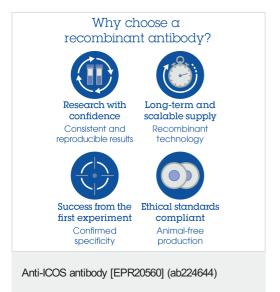


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ICOS antibody
[EPR20560] (ab224644)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling ICOS with ab224644 at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic and membranous staining in a population of lymphoid cells of rat spleen is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



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