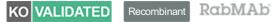
abcam

Product datasheet

Anti-Galectin 3 antibody [EPR19244] ab209344





★★★★★ 5 Abreviews 5 References 13 Images

Overview

Product name Anti-Galectin 3 antibody [EPR19244]

Description Rabbit monoclonal [EPR19244] to Galectin 3

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IP, ICC/IF, IHC-P, WB

Species reactivity Reacts with: Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, SW480, MCF7, A431 and Wild-type A549 whole cell lysates; human heart, kidney and

> stomach lysates. IHC-P: Human liver, stomach, diffuse large B cell lymphoma and colon cancer tissues. ICC/IF: HeLa and A431 cells. Flow Cyt (intra): HeLa cells. IP: 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 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 EPR19244

Isotype IgG

Applications

The Abpromise guarantee

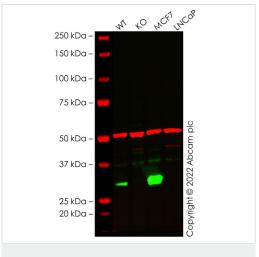
Our <u>Abpromise guarantee</u> covers the use of ab209344 in the following tested applications.

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

| Application | Abreviews | Notes |
|------------------|------------------|--|
| Flow Cyt (Intra) | | 1/50. |
| IP | | 1/30. |
| ICC/IF | ★★★★★ (3) | 1/500. |
| IHC-P | ****(1) | 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| WB | ★★★★★ (1) | 1/1000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa). |

| Target | | |
|-----------------------|---|--|
| Function | Galactose-specific lectin which binds IgE. May mediate with the alpha-3, beta-1 integrin the stimulation by CSPG4 of endothelial cells migration. Together with DMBT1, required for terminal differentiation of columnar epithelial cells during early embryogenesis. | |
| Tissue specificity | A major expression is found in the colonic epithelium. It is also abundant in the activated macrophages. | |
| Sequence similarities | Contains 1 galectin domain. | |
| Cellular localization | Nucleus. Cytoplasmic in adenomas and carcinomas. May be secreted by a non-classical secretory pathway and associate with the cell surface. | |

Images



Western blot - Anti-Galectin 3 antibody [EPR19244] (ab209344)

All lanes : Anti-Galectin 3 antibody [EPR19244] (ab209344) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: LGALS3 knockout A549 cell lysate

Lane 3 : MCF7 cell lysate

Lane 4 : LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

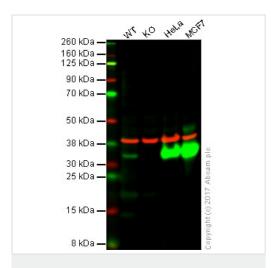
Secondary

All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

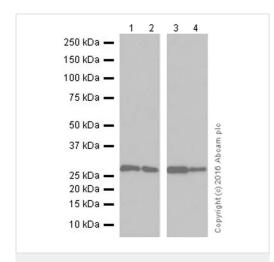
Performed under reducing conditions.

Predicted band size: 26 kDa **Observed band size:** 30 kDa

False colour image of Western blot: Anti-Galectin 3 antibody [EPR19244] 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, ab209344 was shown to bind specifically to Galectin 3. A band was observed at 30 kDa in wild-type A549 cell lysates with no signal observed at this size in LGALS3 knockout cell line. To generate this image, wildtype and LGALS3 knockout A549 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 dilution.



Western blot - Anti-Galectin 3 antibody [EPR19244] (ab209344)



Western blot - Anti-Galectin 3 antibody [EPR19244] (ab209344)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: Galectin 3 (KO) knockout HAP1 whole cell lysate (20 μ g)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: MCF7 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab209344 observed at 35 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab209344 was shown to specifically react with Galectin 3 (KO) in wild-type HAP1 cells. No band was observed when LGALS3 (KO) knockout samples were examined. Wild-type and Galectin 3 (KO) knockout samples were subjected to SDS-PAGE. Ab209344 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.

Lanes 1-2: Anti-Galectin 3 antibody [EPR19244] (ab209344) at 1/10000 dilution

Lanes 3-4: Anti-Galectin 3 antibody [EPR19244] (ab209344) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate

Lane 3: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 4: A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

1/100000 dilution

Predicted band size: 26 kDa Observed band size: 26 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 30 seconds; Lane 3/4: 8 seconds

All lanes: Anti-Galectin 3 antibody [EPR19244] (ab209344) at 1/1000 dilution

Lane 1: Human heart tissue Lane 2: Human kidney tissue Lane 3: Human stomach tissue

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

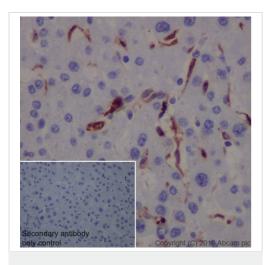
Predicted band size: 26 kDa

Observed band size: 26 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 15 seconds; Lane 3: 30 seconds

Western blot - Anti-Galectin 3 antibody [EPR19244] (ab209344)

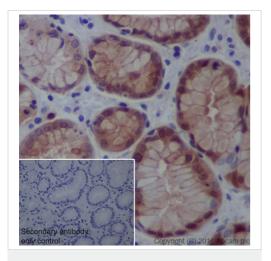


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galectin 3 antibody
[EPR19244] (ab209344)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Galectin 3 with ab209344 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Positive staining on Kupffer cells in the liver is observed. 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.

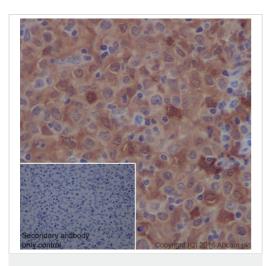


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galectin 3 antibody
[EPR19244] (ab209344)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling Galectin 3 with ab209344 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and nuclear staining on human stomach is observed. 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.

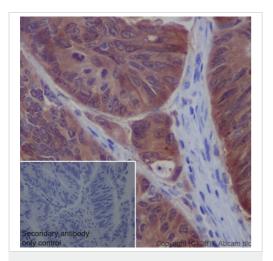


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galectin 3 antibody
[EPR19244] (ab209344)

Immunohistochemical analysis of paraffin-embedded human diffuse large B cell lymphoma tissue labeling Galectin 3 with ab209344 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and weak nuclear staining on human diffuse large B cell lymphoma is observed. 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.

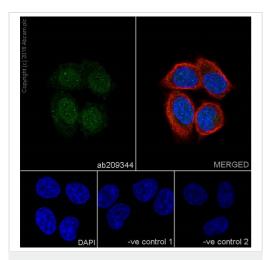


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galectin 3 antibody
[EPR19244] (ab209344)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling Galectin 3 with ab209344 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and weak nuclear staining on human colon cancer is observed. 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-Galectin 3 antibody [EPR19244] (ab209344)

ab209344 MERGED

DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-Galectin 3 antibody [EPR19244] (ab209344)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Galectin 3 with ab209344 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L(Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab209344 at 1/500 dilution followed by Goat Anti-Mouse lgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

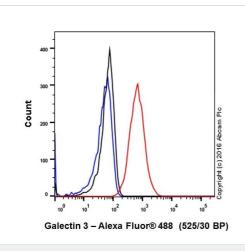
-ve control 2: Anti-alpha Tubulin mouse MAb ($\underline{ab7291}$) at 1/1000 dilution followed by Goat Anti-Rabbit lgG H&L(Alexa Fluor® 488) ($\underline{ab150077}$) secondary antibody at 1/1000 dilution.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cells labeling Galectin 3 with ab209344 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weakly cytoplasmic staining on A431 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

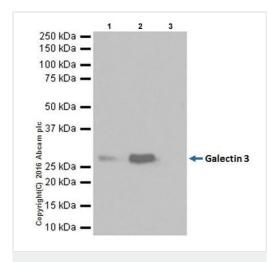
The negative controls are as follows:

-ve control 1: ab209344 at 1/500 dilution followed by ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Galectin 3 antibody [EPR19244] (ab209344)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Galectin 3 with ab209344 at 1/50 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Galectin 3 antibody [EPR19244] (ab209344)

Galectin 3 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab209344 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab209344 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

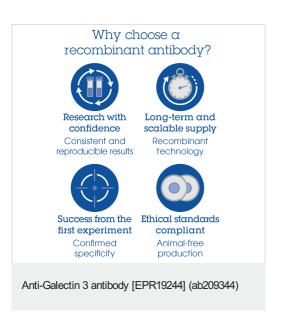
Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab209344 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab209344 in HeLa whole cell lysate.

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

Exposure time: 8 seconds.



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