abcam

Product datasheet

Anti-Galectin 3 antibody [EP2775Y] ab76245

KO VALIDATED Recombinant RabMAb

5 Abreviews 25 References 18 Images

Overview

Product name	Anti-Galectin 3 antibody [EP2775Y]
Description	Rabbit monoclonal [EP2775Y] to Galectin 3
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A375, HeLa, SW480, A431, RAW264.7 and NIH/3T3, C6, Wild-type A549 and MCF7 cell lysates. ICC/IF: Panc-1 and HT-29 cells, RAW264.7 cells and C6 cells . IHC-P: Human lung squamous carcinoma and human thyroid carcinoma tissues. Flow cyt (intra): RAW264.7 cells and C6 cells.
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP2775Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76245 in the following tested applications.

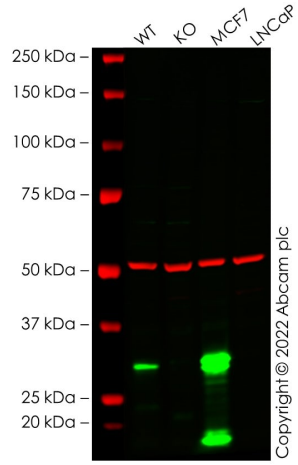
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/1000. **For unpurified use at 1/100 - 1/250.**
WB		1/5000 - 1/10000. Predicted molecular weight: 26 kDa.
IHC-P	★★★★★ **(5)**	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/2000 - 1/5000.**
Flow Cyt (Intra)		1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/1000.

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 [EP2775Y] (ab76245)

All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/5000 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.

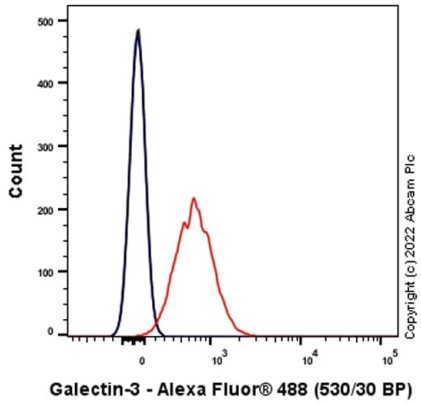
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

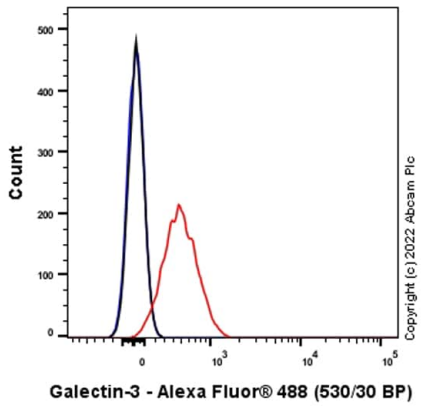
False colour image of Western blot: Anti-Galectin 3 antibody [EP2775Y] staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab76245 was shown to bind specifically to Galectin 3. A band was 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, wild-type 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.



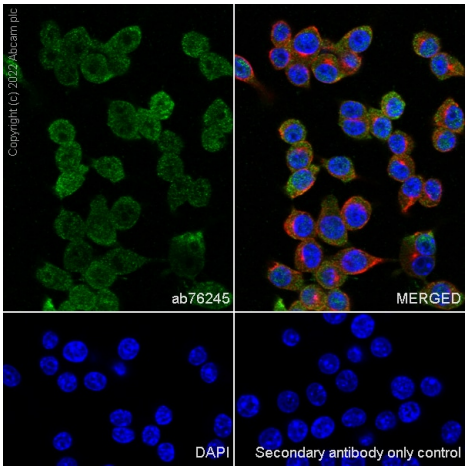
Flow Cytometry (Intracellular) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling Galectin 3 with ab76245 at 1:50 dilution (1µg)/ Red compared with a Rabbit monoclonal IgG (**ab172730**) / Black isotype control and an unlabelled control (Cell without incubation with primary antibody and secondary antibody (Blue)). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

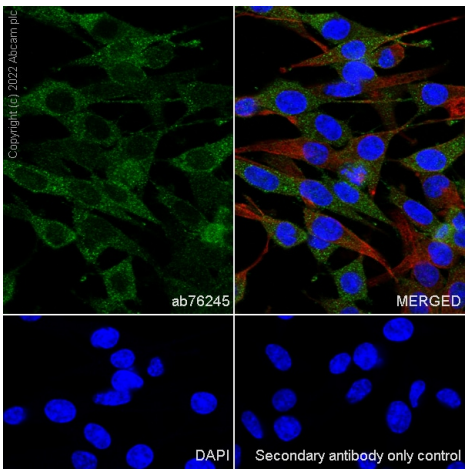
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized C6 (rat glial tumor glial cell) cells labelling Galectin 3 with ab76245 at 1:50 dilution (1µg)/ Red compared with a Rabbit monoclonal IgG (**ab172730**) / Black isotype control and an unlabelled control (Cell without incubation with primary antibody and secondary antibody (Blue)). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 cells labeling Galectin 3 with ab76245 at 1/100 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody at 1/1000 dilution. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution. The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.

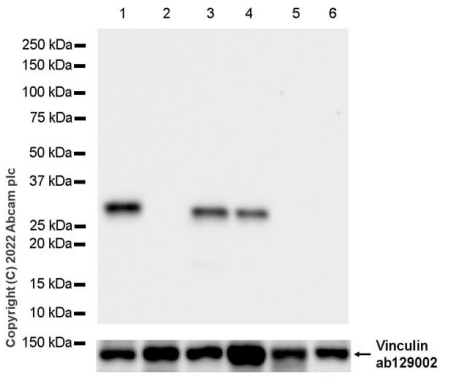
Confocal image showing nuclear and cytoplasmic staining in RAW 264.7 cell line.



Immunocytochemistry/ Immunofluorescence - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized C6 cells labeling Galectin 3 with ab76245 at 1/100 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody at 1/1000 dilution. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution. The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.

Confocal image showing cytoplasmic and weak nuclear staining in C6 cell line.



Western blot - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/1000 dilution

Lane 1 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate
Lane 3 : A431 (human epidermoid carcinoma epithelial cell) whole cell lysate
Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 5 : SK-N-MC (human brain epithelial cell) whole cell lysate
Lane 6 : LNCaP (human prostate carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

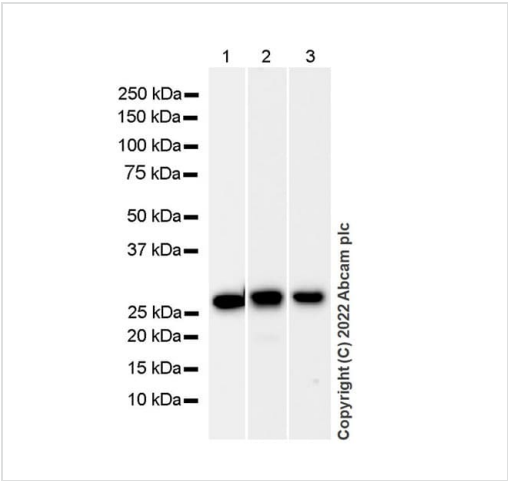
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 26 seconds

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

ab129002 was used as a loading control for Vinculin.



Western blot - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/1000 dilution

Lane 1 : C6 (rat glial tumor glial cell) whole cell lysate
Lane 2 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

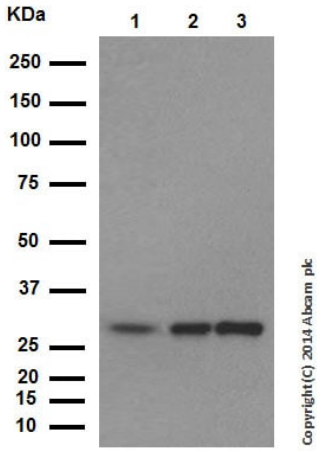
Lysates/proteins at 20 µg per lane.

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

Exposure time: Lane 1: 180 seconds, Lanes 2 and 3: 15 seconds.



Western blot - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/10000 dilution (purified)

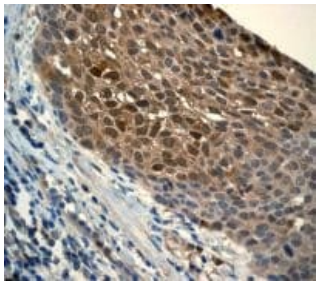
Lane 1 : A375 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 26 kDa
Observed band size: 31 kDa

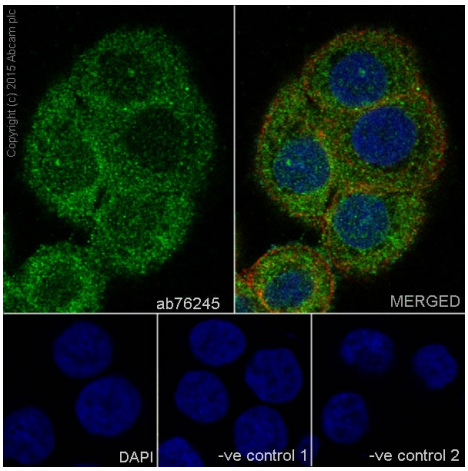
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung squamous carcinoma tissue labelling Galectin 3 with unpurified ab76245.

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

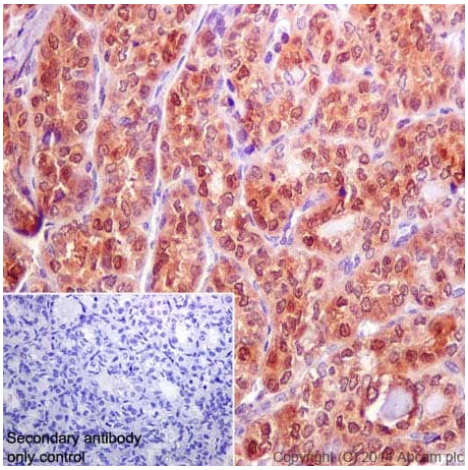


Immunocytochemistry/ Immunofluorescence - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Immunocytochemistry/Immunofluorescence analysis of HT-29 cells labelling Galectin with purified ab76245 at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/500) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

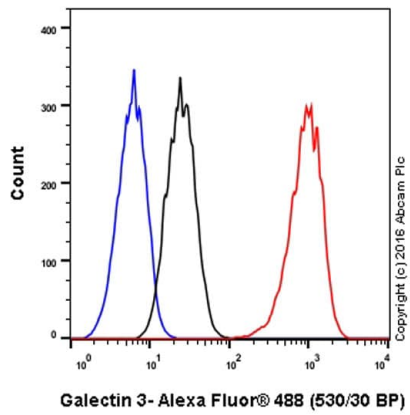
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



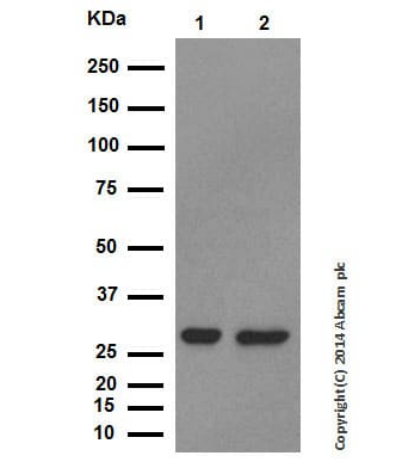
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue labelling Galectin 3 with purified ab76245 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Flow Cytometry (Intracellular) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Galectin 3 with purified ab76245 at 1/50 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/10000 dilution (purified)

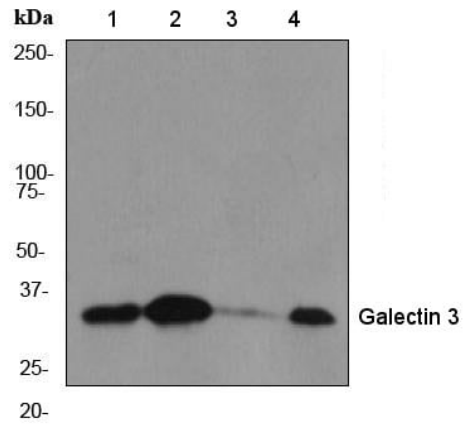
Lane 1 : RAW264.7 cell lysate
Lane 2 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 26 kDa
Observed band size: 31 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

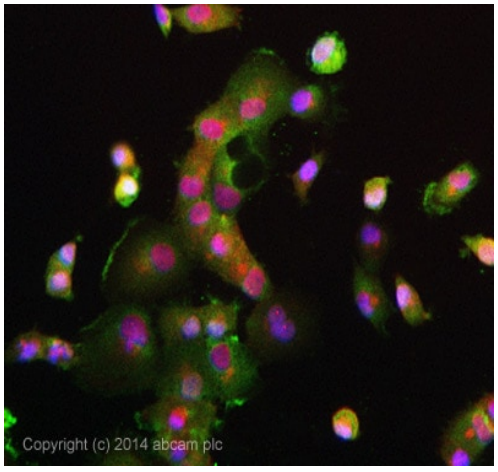
All lanes : Anti-Galectin 3 antibody [EP2775Y] (ab76245) at 1/10000 dilution (unpurified)

Lane 1 : A375 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : SW480 cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 10 µg per lane.

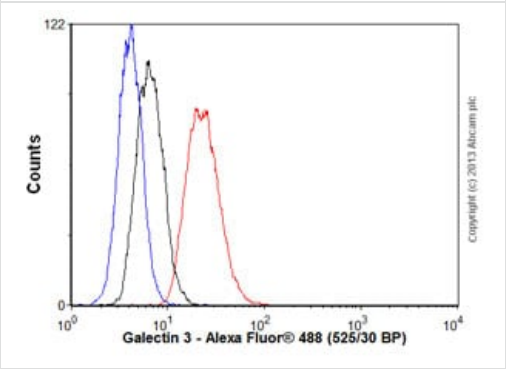
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 26 kDa
Observed band size: 31 kDa



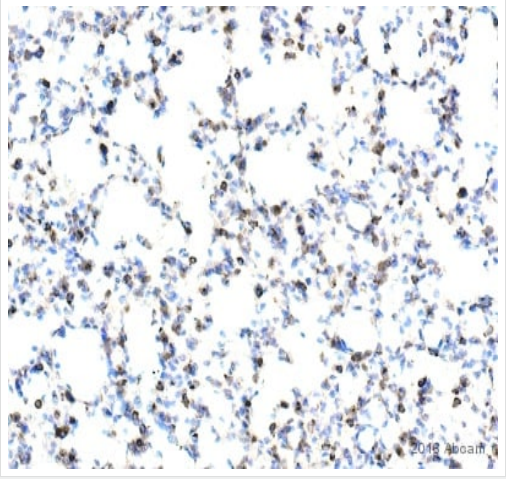
Immunocytochemistry/ Immunofluorescence - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

ICC/IF image of unpurified ab76245 stained Panc-1 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab76245 at 10µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti-rabbit (**ab150081**) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Flow Cytometry (Intracellular) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

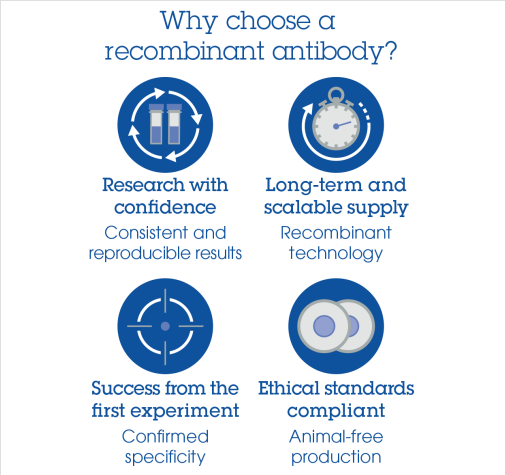
Overlay histogram showing THP1 cells stained with unpurified ab76245 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% human serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76245, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Galectin 3 antibody [EP2775Y] (ab76245)

This image is courtesy of an abreview submitted by Carl Hobbs

Immunohistochemical analysis of formaldehyde fixed mouse lung tissue sections labelling Galectin 3 with ab76245 at a dilution of 1/6000. The secondary antibody used was biotin conjugated goat anti rabbit IgG at a dilution of 1/300. Antigen retrieval was heat mediated using citric acid.



Anti-Galectin 3 antibody [EP2775Y] (ab76245)

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