abcam

Product datasheet

Anti-galectin 9/Gal-9 antibody [EPR22214] ab227046

敲除验证 重组 RabMAb

4 References 10 图像

概述

产品名称	Anti-galectin 9/Gal-9抗体[EPR22214]
描述	兔单克隆抗体[EPR22214] to galectin 9/Gal-9
宿主	Rabbit
特异性	This antibody is not recommended for mouse and rat in IHC.
经测试应用	**适用于:** ICC/IF, Flow Cyt (Intra), WB, IHC-P **不适用于:** IP
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	IHC-P: Human liver and colon tissue. WB: U937, HL-60 and THP-1 whole cell lysates. ICC/IF: U937 cells. Flow Cyt (intra): U937 cells.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR22214
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab227046于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

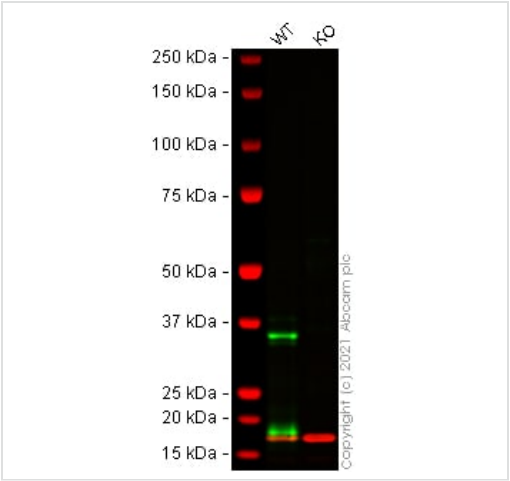
应用	Ab评论	说明
ICC/IF		1/250.
Flow Cyt (Intra)		1/500.
WB		1/1000. Predicted molecular weight: 40 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

应用说明	Is unsuitable for IP.

靶标

功能	Binds galactosides. Has high affinity for the Forssman pentasaccharide. May play a role in thymocyte-epithelial interactions relevant to the biology of the thymus. Inhibits cell proliferation. It is a ligand for HAVCR2/TIM3. Induces T-helper type 1 lymphocyte (Th1) death. Isoform Short acts as an eosinophil chemoattractant.
组织特异性	Peripheral blood leukocytes and lymphatic tissues. Overexpressed in Hodgkin disease tissue.
序列相似性	Contains 2 galectin domains.
结构域	Contains two homologous but distinct carbohydrate-binding domains.
细胞定位	Cytoplasm. Secreted. May also be secreted by a non-classical secretory pathway.

图片



Western blot - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

All lanes : Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : LGALS9 knockout THP-1 cell lysate

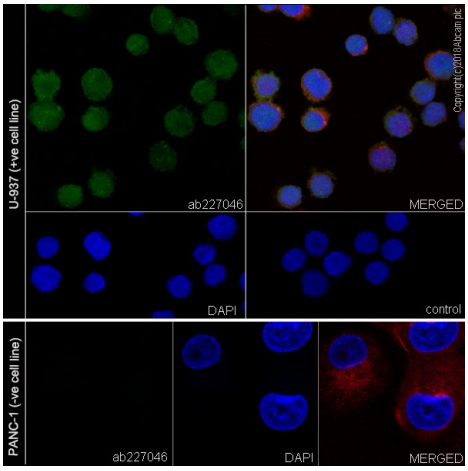
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 40 kDa
Observed band size: 35 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab227046 observed at 35 kDa. Red - loading control Mouse anti Histone H3 observed at 18 kDa.

ab227046 was shown to react with galectin 9/Gal-9 in wild-type THP-1 cells in Western blot with loss of signal observed in LGALS9 knockout cell line **ab269505** (knockout cell lysate **ab269667**). Wild-type THP-1 and LGALS9 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab227046 and Mouse anti Histone H3 overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

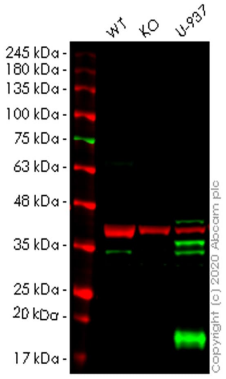


Immunocytochemistry/ Immunofluorescence - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

Immunocytochemistry analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Cell line U937 (human histiocytic lymphoma cell line) cells labeling galectin 9/Gal-9 with ab227046 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). The nuclear counterstain is DAPI (blue).
Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).

Confocal image showing nuclear and cytoplasmic staining in U937 cell line.

Negative control: PANC-1.



Western blot - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

All lanes : Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046) at 1/500 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : Galectin 9 knockout A549 cell lysate
Lane 3 : U-937 cell lysate

Lysates/proteins at 20 µg per lane.

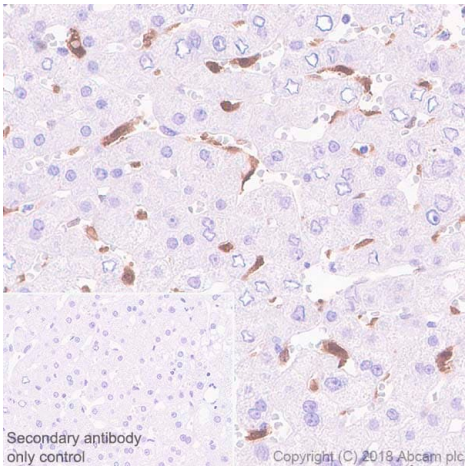
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 40 kDa
Observed band size: 34-39 kDa

Lanes 1-4: Merged signal (red and green). Green - ab227046 observed at 34-39 kDa. Red - loading control **ab8245** observed at 36 kDa.

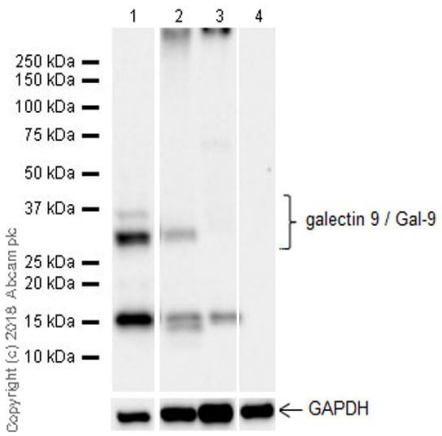
ab227046 Anti-galectin 9/Gal-9 antibody [EPR22214] was shown to specifically react with galectin 9/Gal-9 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab266923** (knockout cell lysate **ab256976**) was used. Wild-type and galectin 9/Gal-9 knockout samples were subjected to SDS-PAGE. ab227046 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-

Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling galectin 9/Gal-9 with ab227046 at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear and cytoplasmic staining on Kupffer cells of human liver (PMID: 20209097, PMID: 18202194) is observed. Counterstained 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 **ab93684** (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

All lanes : Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046) at 1/1000 dilution

Lane 1 : U937 (human histiocytic lymphoma monocyte), whole cell lysate
Lane 2 : HL-60 (human Acute Promyelocytic Leukemia promyeloblast), whole cell lysate
Lane 3 : MOLT-4 (human lymphoblastic leukemia T lymphoblast), whole cell lysate
Lane 4 : PANC-1 (human pancreatic epithelioid 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 1/20000 dilution

Predicted band size: 40 kDa
Observed band size: 34-39 kDa
Additional bands at: 13-15 kDa (possible degradation product)

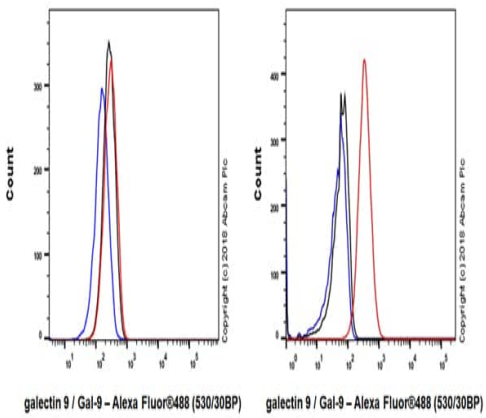
Exposure time: 59 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression of the isoforms observed is consistent with what has been described in the literature (PMID 11886844, PMID: 22805533).

Degraded fragments (13-15 kDa) have also been descried (PMID: 15811318).

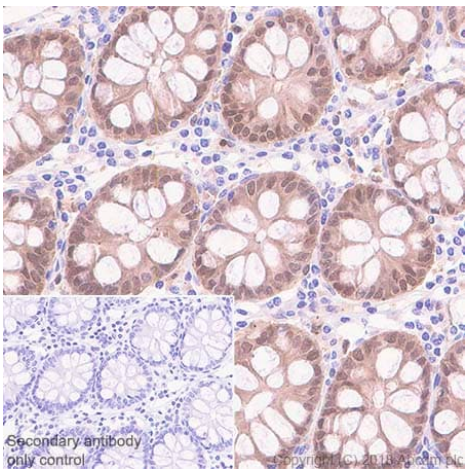
Negative control: PANC-1.



Flow Cytometry (Intracellular) - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

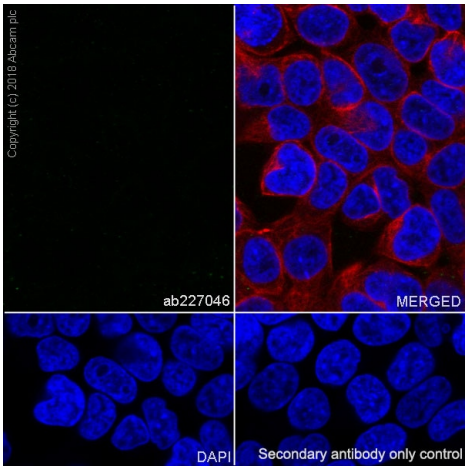
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized PANC-1 (human pancreatic epithelial cancinoma cell line) (Left) / U937 (human histiocytic lymphoma cell line) (Right) cell line labeling galectin 9/Gal-9 with ab227046 at 1/500 (red) compared with a Rabbit monoclonal IgG (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

Negative Control - PANC-1 cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling galectin 9/Gal-9 with ab227046 at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear and cytoplasmic staining on epithelial cells of human colon (PMID: 18202194) is observed. Counterstained 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 **ab93684** (Tris/EDTA buffer, pH 9.0).

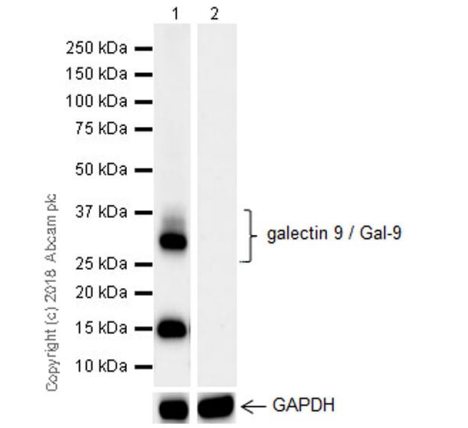


Immunocytochemistry/ Immunofluorescence - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human epithelial cell line from embryonic kidney) cells labeling galectin 9/Gal-9 with ab227046 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing no staining in HEK-293T cells

Negative control: HEK-293T (PMID: 11698107). The nuclear counterstain is DAPI (blue).
Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).
Secondary antibody only control: Used PBS instead of the primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046)

All lanes : Anti-galectin 9/Gal-9 antibody [EPR22214] (ab227046) at 1/1000 dilution

Lane 1 : THP-1 (human monocytic leukemia monocyte), whole cell lysate
Lane 2 : HEK-293T (human embryonic kidney epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 40 kDa
Observed band size: 34-39 kDa
Additional bands at: 13-15 kDa (possible degradation product)

Exposure time: 59 seconds

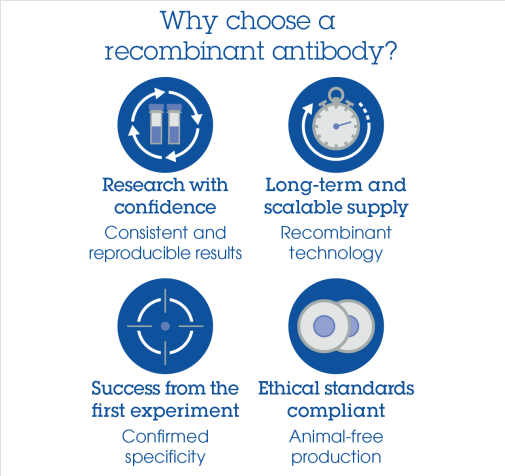
Blocking/Dilution buffer: 5% NFDM/TBST.

The expression of the isoforms observed is consistent with what has been described in the literature (PMID 11886844, PMID: 22805533).

Degraded fragments (13-15 kDa) has also been described (PMID:

15811318).

Negative control: HEK-293T (PMID: 24333696).



Anti-galectin 9/Gal-9 antibody [EPR22214]

(ab227046)

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