abcam

Product datasheet

Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free ab224264

KO VALIDATED Recombinant RabMAb

1 References 6 Images

Overview

Product name	Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR19912] to Lipocalin-2 / NGAL - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** 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: SW480 and A431 whole cell lysates; human ovary cancer, fetal spleen and colon cancer lysates; IHC-P: Human spleen, liver and ovary cancer tissues.
General notes	ab224264 is the carrier-free version of **ab206427**. 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 cell-based 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19912
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab224264 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		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. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).

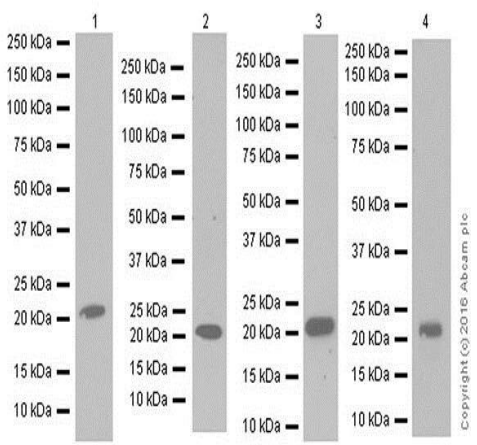
Target

Function Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development. Binds iron through association with 2,5-dihydroxybenzoic acid (2,5-DHBA), a siderophore that shares structural similarities with bacterial enterobactin, and delivers or removes iron from the cell, depending on the context. Iron-bound form (holo-24p3) is internalized following binding to the SLC22A17 (24p3R) receptor, leading to release of iron and subsequent increase of intracellular iron concentration. In contrast, association of the iron-free form (apo-24p3) with the SLC22A17 (24p3R) receptor is followed by association with an intracellular siderophore, iron chelation and iron transfer to the extracellular medium, thereby reducing intracellular iron concentration. Involved in apoptosis due to interleukin-3 (IL3) deprivation: iron-loaded form increases intracellular iron concentration without promoting apoptosis, while iron-free form decreases intracellular iron levels, inducing expression of the proapoptotic protein BCL2L11/BIM, resulting in apoptosis. Involved in innate immunity, possibly by sequestrating iron, leading to limit bacterial growth.

Tissue specificity Expressed in bone marrow and in tissues that are prone to exposure to microorganism. High

	expression is found in bone marrow as well as in uterus, prostate, salivary gland, stomach, appendix, colon, trachea and lung. Not found in the small intestine or peripheral blood leukocytes.
Sequence similarities	Belongs to the calycin superfamily. Lipocalin family.
Cellular localization	Secreted. Upon binding to the SLC22A17 (24p3R) receptor, it is internalized.

Images



Western blot - Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free (ab224264)

All lanes : Anti-Lipocalin-2 / NGAL antibody [EPR19912] (**ab206427**) at 1/1000 dilution

Lane 1 : A431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : Human ovary cancer lysate
Lane 3 : Human fetal spleen lysate
Lane 4 : Human colon cancer lysate

Lysates/proteins at 10 µg per lane.

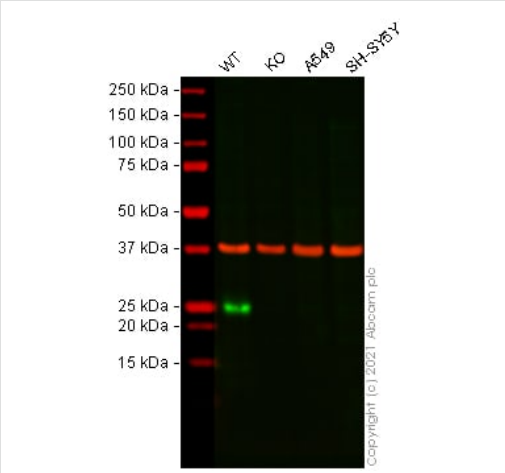
Secondary
Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution
Lanes 2-4 : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 22 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab206427**).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 3 minutes; Lane 4: 30 seconds.



Western blot - Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free (ab224264)

All lanes : Anti-Lipocalin-2 / NGAL antibody [EPR19912] (**ab206427**) at 1/1000 dilution

Lane 1 : Wild-type SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 2 : LCN2 knockout SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

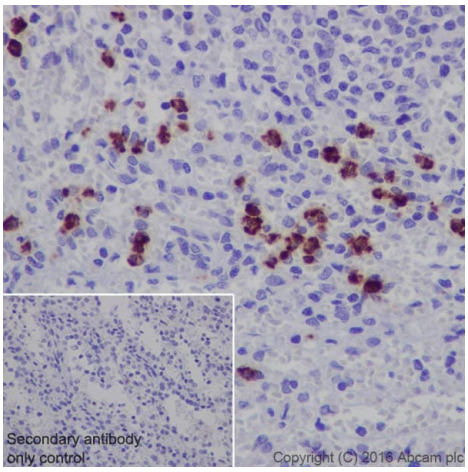
Predicted band size: 22 kDa
Observed band size: 25 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab206427**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab206427** observed at 25 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab206427 was shown to react with Lipocalin-2 / NGAL in wild-type SW480 cells in Western blot with loss of signal observed in LCN2 knockout cell line **ab270486** (knockout cell lysate **ab270509**). Wild-type SW480 and LCN2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab206427** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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.



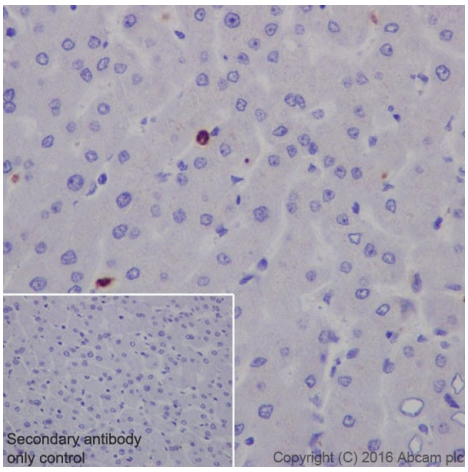
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free (ab224264)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Lipocalin-2 / NGAL with **ab206427** at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Positive staining on neutrophils is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab206427**).

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



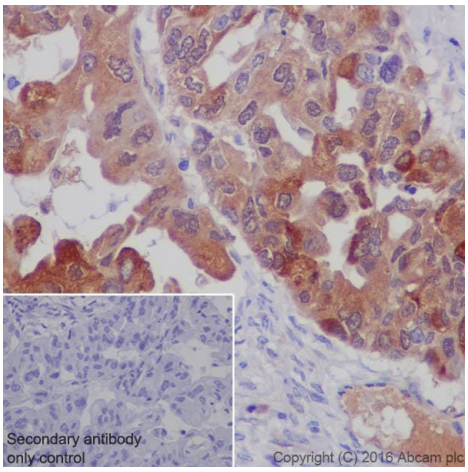
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free (ab224264)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Lipocalin-2 / NGAL with **ab206427** at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Positive staining on neutrophils and negative on hepatocytes. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab206427**).

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



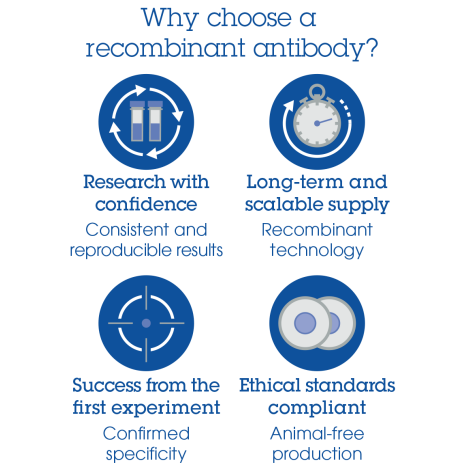
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lipocalin-2 / NGAL antibody [EPR19912] - Low endotoxin, Azide free (ab224264)

This IHC data was generated using the same anti-NGAL antibody clone [EPR19912] in a different buffer formulation (cat# **ab20627**).

Immunohistochemical analysis of paraffin-embedded human ovary cancer tissue labeling Lipocalin-2 / NGAL with **ab206427** at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasmic staining on human ovary cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.



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