abcam

Product datasheet

Anti-MUC1 antibody [EPR1023] ab109185





重组 RabMAb

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概述

产品名称 Anti-MUC1抗体[EPR1023]

描述 兔单克隆抗体[EPR1023] to MUC1

宿主 Rabbit

特异性 This antibody detects the 17 kDa carboxy-terminal subunit (subunit beta). Depending on the N-

glycosylation extent, the size of this subunit is estimated to be between 17 kDa (without N-

glycosylation) and 23-25 kDa

适用于: Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P 经测试应用

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 T47-D cell lysate, colon cancer lysate, Human breast carcinoma tissue, Human normal breast

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 -20°C. Stable for 12 months at -20°C.

 $K_D = 8.90 \times 10^{-12} M$ 解离常数(K_□)

> 10⁻¹² LOW HIGH AFFINITY **AFFINITY**

Learn more about K_D

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR1023

同种型 IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab109185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
Flow Cyt (Intra)		1/30. For unpurified use at 1/100 - 1/1000. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/1000. For unpurified use at 1/100 - 1/250.
WB		1/1000 - 1/5000. Predicted molecular weight: 17 kDa.
IP		1/20. For unpurified use at 1/10 - 1/100.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

靶标

功能

The alpha subunit has cell adhesive properties. Can act both as an adhesion and an antiadhesion protein. May provide a protective layer on epithelial cells against bacterial and enzyme attack.

The beta subunit contains a C-terminal domain which is involved in cell signaling, through phosphorylations and protein-protein interactions. Modulates signaling in ERK, SRC and NF-kappa-B pathways. In activated T-cells, influences directly or indirectly the Ras/MAPK pathway. Promotes tumor progression. Regulates TP53-mediated transcription and determines cell fate in the genotoxic stress response. Binds, together with KLF4, the PE21 promoter element of TP53 and represses TP53 activity.

组织特异性

Expressed on the apical surface of epithelial cells, especially of airway passages, breast and uterus. Also expressed in activated and unactivated T-cells. Overexpressed in epithelial tumors, such as breast or ovarian cancer and also in non-epithelial tumor cells. Isoform Y is expressed in tumor cells only.

疾病相关

MUC1/CA 15-3 is used as a serological clinical marker of breast cancer to monitor response to

breast cancer treatment and disease recurrence (PubMed:20816948). Decreased levels over time may be indicative of a positive response to treatment. Conversely, increased levels may indicate disease progression. At an early stage disease, only 21% of patients exhibit high MUC1/CA 15-3 levels, that is why CA 15-3 is not a useful screening test. Most antibodies target the highly immunodominant core peptide domain of 20 amino acid (APDTRPAPGSTAPPAHGVTS) tandem repeats. Some antibodies recognize glycosylated enitones

Medullary cystic kidney disease 1

Contains 1 SEA domain.

During fetal development, expressed at low levels in the colonic epithelium from 13 weeks of gestation.

Highly glycosylated (N- and O-linked carbohydrates and sialic acid). O-glycosylated to a varying degree on serine and threonine residues within each tandem repeat, ranging from mono- to penta-glycosylation. The average density ranges from about 50% in human milk to over 90% in T47D breast cancer cells. Further sialylation occurs during recycling. Membrane-shed glycoproteins from kidney and breast cancer cells have preferentially sialyated core 1 structures, while secreted forms from the same tissues display mainly core 2 structures. The O-glycosylated content is overlapping in both these tissues with terminal fucose and galactose, 2- and 3-linked galactose, 3- and 3,6-linked GalNAc-ol and 4-linked GlcNAc predominating. Differentially O-glycosylated in breast carcinomas with 3,4-linked GlcNAc. N-glycosylation consists of highmannose, acidic complex-type and hybrid glycans in the secreted form MUC1/SEC, and neutral complex-type in the transmembrane form, MUC1/TM.

Proteolytic cleavage in the SEA domain occurs in the endoplasmic reticulum by an autoproteolytic mechanism and requires the full-length SEA domain as well as requiring a Ser, Thr or Cys residue at the P + 1 site. Cleavage at this site also occurs on isoform MUC1/X but not on isoform MUC1/Y. Ectodomain shedding is mediated by ADAM17.

Dual palmitoylation on cysteine residues in the CQC motif is required for recycling from endosomes back to the plasma membrane.

Phosphorylated on tyrosines and serine residues in the C-terminal. Phosphorylation on tyrosines in the C-terminal increases the nuclear location of MUC1 and beta-catenin. Phosphorylation by PKC delta induces binding of MUC1 to beta-catenin/CTNNB1 and thus decreases the formation of the beta-catenin/E-cadherin complex. Src-mediated phosphorylation inhibits interaction with GSK3B. Src- and EGFR-mediated phosphorylation on Tyr-1229 increases binding to beta-catenin/CTNNB1. GSK3B-mediated phosphorylation on Ser-1227 decreases this interaction but restores the formation of the beta-cadherin/E-cadherin complex. On T-cell receptor activation, phosphorylated by LCK. PDGFR-mediated phosphorylation increases nuclear colocalization of MUC1CT and CTNNB1.

The N-terminal sequence has been shown to begin at position 24 or 28.

Secreted; Cell membrane. Cytoplasm. Nucleus. On EGF and PDGFRB stimulation, transported to the nucleus through interaction with CTNNB1, a process which is stimulated by phosphorylation. On HRG stimulation, colocalizes with JUP/gamma-catenin at the nucleus and Apical cell membrane. Exclusively located in the apical domain of the plasma membrane of highly polarized epithelial cells. After endocytosis, internalized and recycled to the cell membrane. Located to microvilli and to the tips of long filopodial protusions.

序列相似性

发展阶段

翻译后修饰

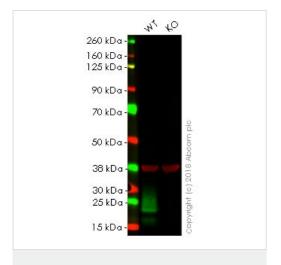
细胞定位

图片



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUC1 antibody
[EPR1023] (ab109185)

Immunohistochemical staining of paraffin embedded human endometrium with purified ab109185 at a working dilution of 1 in 500. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-MUC1 antibody [EPR1023] (ab109185)

All lanes : Anti-MUC1 antibody [EPR1023] (ab109185) at 1/1000 dilution

Lane 1: Wild-type HeLa whole cell lysate

Lane 2: MUC1 knockout HeLa whole cell lysate

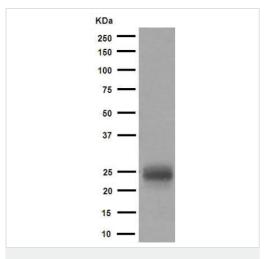
Lysates/proteins at 20 µg per lane.

Predicted band size: 17 kDa

Observed band size: 17-24 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab109185 observed at 24 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab109185 was shown to specifically react with MUC1 in wild-type HeLa cells as signal was lost in MUC1 knockout cells. Wild-type and MUC1 knockout samples were subjected to SDS-PAGE. Ab109185 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-MUC1 antibody [EPR1023] (ab109185)

Anti-MUC1 antibody [EPR1023] (ab109185) at 1/1000 dilution (Purified antibody) + Colon cancer at 10 µg

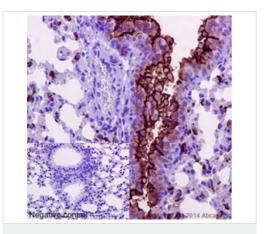
Secondary

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

Predicted band size: 17 kDa Observed band size: 24 kDa

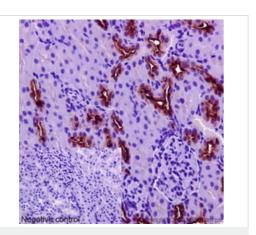
Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

Immunohistochemical staining of paraffin embe



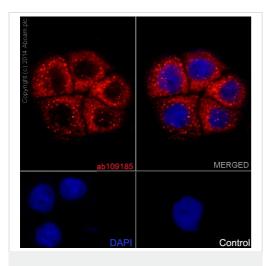
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUC1 antibody
[EPR1023] (ab109185)

Immunohistochemical staining of paraffin embedded mouse lung with purified ab109185 at a working dilution of 1 in 500. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



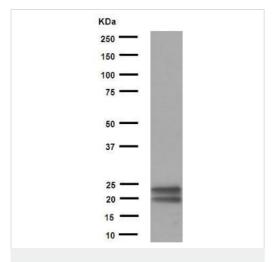
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUC1 antibody
[EPR1023] (ab109185)

Immunohistochemical staining of paraffin embedded rat kidney with purified ab109185 at a working dilution of 1 in 500. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-MUC1 antibody [EPR1023] (ab109185)

Immunofluorescence staining of A431 cells with purified ab109185 at a working dilution of 1 in 1000, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 555 goat anti rabbit, used at a dilution of 1 in 400. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, purified ab109185 was used at a dilution of 1/200 followed by an Alexa Fluor[®] 555 goat anti-mouse antibody at a dilution of 1/500.



Western blot - Anti-MUC1 antibody [EPR1023] (ab109185)

Anti-MUC1 antibody [EPR1023] (ab109185) at 1/5000 dilution (Purified) + T47D cell lysate at 10 μg

Secondary

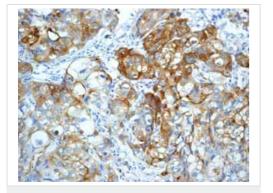
Secondary ab: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 17 kDa

Observed band size: 18-25 kDa

Blocking buffer: 5% NFDM/TBST

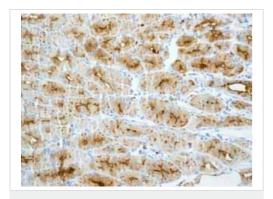
Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUC1 antibody
[EPR1023] (ab109185)

ab109185 (unpurified) showing positive staining in Breast ductal infiltrating carcinoma tissue.

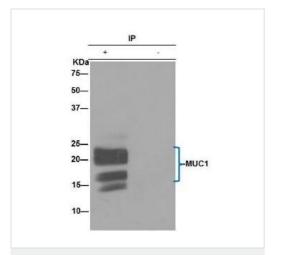
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUC1 antibody
[EPR1023] (ab109185)

ab109185 (unpurified) showing positive staining in Normal stomach tissue

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

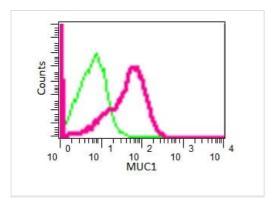


Immunoprecipitation - Anti-MUC1 antibody [EPR1023] (ab109185)

ab109185 (purified) at 1/20 immunoprecipitating MUC1 in T47-D (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500).

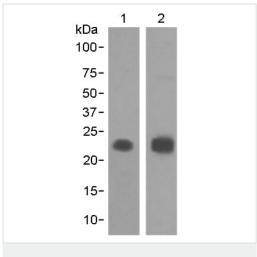
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

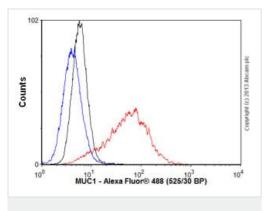


Flow Cytometry (Intracellular) - Anti-MUC1 antibody [EPR1023] (ab109185)

Overlay histogram showing MCF7 cells fixed in 2% PFA and stained with purified ab 109185 at a dilution of 1 in 30 (pink line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control.



Western blot - Anti-MUC1 antibody [EPR1023] (ab109185)



Flow Cytometry (Intracellular) - Anti-MUC1 antibody [EPR1023] (ab109185)

All lanes : Anti-MUC1 antibody [EPR1023] (ab109185) at 1/1000 dilution (Unpurified)

Lane 1: T47-D cell lysate

Lane 2: Human colon cancer lysate

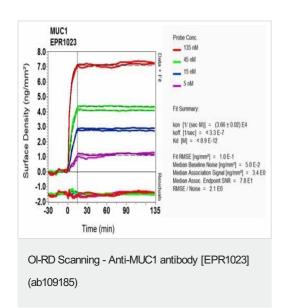
Lysates/proteins at 10 µg per lane.

Performed under reducing conditions.

Predicted band size: 17 kDa

Additional bands at: 17 kDa (possible cleavage fragment)

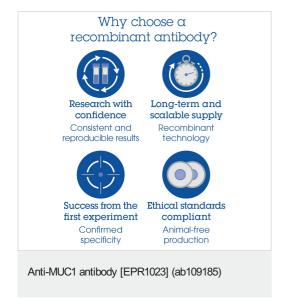
Overlay histogram showing MCF7 cells stained with unpurified ab109185 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109185, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) $(0.1\mu g/1x10^6 \text{ cells})$ 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. This antibody gave a positive signal in MCF7 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Equilibrium disassociation constant (K_D) of unpurified ab109185.

Learn more about K_D

Click here to learn more about K_D



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