**Product datasheet**

**Anti-MUC1 antibody [EP1024Y] ab45167**

- **Product name**: Anti-MUC1 antibody [EP1024Y]
- **Description**: Rabbit monoclonal [EP1024Y] to MUC1
- **Host species**: Rabbit
- **Specificity**: Based on the immunogen sequence, the antibody recognises several isoforms of MUC1 (Uniprot ID P15941). They are Isoform Y (28 kDa), Isoform Y-LSP (28 kDa), Isoform S2 (17 kDa) and Isoform J13 (28 kDa).
- **Tested applications**: Suitable for: IHC-Fr, WB, Flow Cyt, IP, ICC/IF
- **Species reactivity**: Reacts with: Mouse, Rat, Human
- **Immunogen**: Synthetic peptide corresponding to Human MUC1 aa 1-100 (N terminal).
- **Positive control**: T47D, MCF7 and A549 cell lysates, human kidney, human breast carcinoma, human thyroid carcinoma, human colon cancer lysate, human fetal lung lysate, rat liver lysate and mouse liver lysate.
- **General notes**: Isoform 7 of MUC1 behaves as a receptor and binds the secreted isoform 5. The binding induces the phosphorylation of the isoform 7, alters cellular morphology and initiates cell signaling. The mouse and rat recommendation is based on WB results. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

*We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.*

**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

Primary antibody notes
Isoform 7 of MUC1 behaves as a receptor and binds the secreted isoform 5. The binding induces the phosphorylation of the isoform 7, alters cellular morphology and initiates cell signaling.

Clonality
Monoclonal

Clone number
EP1024Y

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab45167 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>1/1000. Detects a band of approximately 27 kDa. MUC1 isoform 7.</td>
<td></td>
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<tr>
<td>Flow Cyt</td>
<td>1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IP</td>
<td>1/20. For unpurified use at 1/50.</td>
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<tr>
<td>ICC/IF</td>
<td>1/500.</td>
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Target

Function
The alpha subunit has cell adhesive properties. Can act both as an adhesion and an anti-adhesion 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.

Tissue specificity
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.

Involvement in disease
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 (APDTRPAGSTAPPAHGVTS) tandem repeats. Some antibodies recognize glycosylated epitopes.

Medullary cystic kidney disease 1

<table>
<thead>
<tr>
<th>Sequence similarities</th>
<th>Contains 1 SEA domain.</th>
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<tbody>
<tr>
<td>Developmental stage</td>
<td>During fetal development, expressed at low levels in the colonic epithelium from 13 weeks of gestation.</td>
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<tr>
<td>Post-translational modifications</td>
<td>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 sialylated 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 6-linked GalNAc-ol and 4-linked GlcNAc predominating. Differentially O-glycosylated in breast carcinomas with 3,4-linked GlcNAc. N-glycosylation consists of high-mannose, 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.</td>
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<tr>
<td>Cellular localization</td>
<td>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 protrusions.</td>
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<td>Images</td>
<td>3</td>
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</table>
Unpurified ab45167 staining MUC1 on E14.5 mouse gut epithelium by IHC-Fr. The tissue was formaldehyde fixed and then blocked for 1 hour at 25°C. The primary antibody was diluted 1/400 and incubated with the sample for 16 hours at 4°C. An Alexa Fluor 488 conjugated goat anti-rabbit antibody was used as the secondary.

Immunocytochemistry/Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling purified MUC1 with ab45167 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Nuclei counterstained with DAPI (blue).

Confocal image showing membranous staining on MCF7 cell line.

**Negative control**: HCT-116 (PMID: 14998492).
Flow cytometry analysis of T47D (human mammary gland ductal carcinoma) cells labelling MUC1 with unpurified ab45167 (pink) at a dilution of 1/150. Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG was used as the secondary antibody. Rabbit monoclonal IgG (ab172730) was used as the isotype control (green).

**Western blot**

**All lanes**: Anti-MUC1 antibody [EP1024Y] (ab45167) at 1/1000 dilution (purified)

- **Lane 1**: Human colon cancer lysate
- **Lane 2**: Rat liver lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

**Observed band size**: 27 kDa

*why is the actual band size different from the predicted?*

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
**Immunoprecipitation - Anti-MUC1 antibody [EP1024Y] (ab45167)**

Lane 1 (input): Human fetal lung lysate 10ug
Lane 2 (+): ab45167 + Human fetal lung lysate 10ug
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab45167 in Human fetal lung lysate

For western blotting, ab131366 VeriBlot for IP Detection Reagent (HRP) was used for detection (1/1000).

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

**Flow Cytometry - Anti-MUC1 antibody [EP1024Y] (ab45167)**

Flow Cytometry analysis of A549 (human lung carcinoma cell line) cells labeling MUC1 with purified ab45167 at 1/20 dilution (10 ug/ml). Cells were fixed with 4% paraformaldehyde. A goat anti-rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody at 1/2000 dilution. Black - Isotype control, Rabbit monoclonal IgG. Blue - unlabeled control, cells without incubation with primary antibody and secondary antibody.

**Western blot - Anti-MUC1 antibody [EP1024Y] (ab45167)**

Anti-MUC1 antibody [EP1024Y] (ab45167) at 1/5000 dilution (purified) + Mouse liver lysate at 20 µg

Secondary
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

**Observed band size:** 27 kDa *why is the actual band size different from the predicted?*

Blocking and diluting buffer and concentration: 5% NFDM /TBST.
Anti-MUC1 antibody [EP1024Y] (ab45167) at 1/2000 dilution (unpurified) + T47D cell lysate at 10 µg

**Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

**Observed band size:** 27 kDa *why is the actual band size different from the predicted?*

**Exposure time:** 3 minutes

Blocking and diluting buffer: 5% NFDM/TBST.

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