# Product datasheet

## Anti-MUC1 antibody [HMFG1 (aka 1.10.F3)] ab70475

<table>
<thead>
<tr>
<th>Overview</th>
<th></th>
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<tbody>
<tr>
<td><strong>Product name</strong></td>
<td>Anti-MUC1 antibody [HMFG1 (aka 1.10.F3)]</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Mouse monoclonal [HMFG1 (aka 1.10.F3)] to MUC1</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: WB, IHC-P, ICC</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Human</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Other Immunogen Type corresponding to Human MUC1. Delipidated human milk fat globule membrane.</td>
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<tr>
<td><strong>Epitope</strong></td>
<td>This antibody recognizes a peptide epitope (PDTR) within the VNTR region of the extracellular domain of MUC1 (PubMed ID: PMC3021526).</td>
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<tr>
<td><strong>Positive control</strong></td>
<td>IHC-P: Human normal colon FFPE tissue sections. ICC: MCF7 cells. WB: Human breast tissue lysate, human colon tissue lysate and MCF whole cell lysate.</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact <a href="mailto:orders@abcam.com">orders@abcam.com</a> or you can find further information here. For a more comprehensive guide to this epitope of HMFG1 clone, we recommend the following publications; Petrakou E et al. 1998: Epitope Mapping of Anti-MUC1 Mucin Protein Core Monoclonal Antibodies. Tumour Biology. Verhoeyen ME et al. 1993: Construction of a reshaped HMFG1 antibody and comparison of its fine specificity with that of the parent mouse antibody. Immunology. Reproducibility is key to advancing scientific discovery and accelerating scientists’ next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications &amp; species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications &amp; species that this product is Abpromise guaranteed for. We are also updating the applications &amp; species that this product has been “predicted to work</td>
</tr>
</tbody>
</table>
with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer
Preservative: 0.02% Sodium azide
Constituent: PBS

Purity
Protein A purified

Clonality
Monoclonal

Clone number
HMFG1 (aka 1.10.F3)

Myeloma
P3-NS1/1-Ag4-1

Isotype
IgG1

Applications

Our Abpromise guarantee covers the use of ab70475 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>
</thead>
<tbody>
<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Predicted molecular weight: 122 kDa.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
</tr>
<tr>
<td>ICC</td>
<td>★★★★亸亸</td>
<td>Use a concentration of 10 µg/ml.</td>
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</tbody>
</table>

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 (APDTRPAGGSTAPPAHGVTS) tandem repeats. Some antibodies recognize glycosylated epitopes.
Medullary cystic kidney disease 1

**Sequence similarities**
Contains 1 SEA domain.

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

**Post-translational modifications**
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 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 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.

**Cellular localization**
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.
Immunocytochemistry - Anti-MUC1 antibody
HMFG1 (aka 1.10.F3) (ab70475)

Lab

ab70475 staining MUC1 in MCF7 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab70475 at 10µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MUC1 antibody [HMFG1 (aka 1.10.F3)] (ab70475)

IHC image of MUC1 staining in human normal colon formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab70475, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
Western blot - Anti-MUC1 antibody [HMFG1 (aka 1.10.F3)] (ab70475)

All lanes: Anti-MUC1 antibody [HMFG1 (aka 1.10.F3)] (ab70475) at 1 µg/ml

Lane 1: Human breast tissue lysate - total protein (ab30090)

Lane 2: Human colon tissue lysate - total protein (ab30051)

Lane 3: MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 122 kDa

Exposure time: 4 minutes

Ab70475 staining human normal lung. Staining is localised to the apical membrane.

Left panel: with primary antibody at 1 µg/ml. Right panel: isotype control.

Sections were stained using an automated system DAKO Autostainer Plus, at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffers EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and
incubation time (overnight incubation), and amplification may be required.

ab70475 stained MCF7 cells. The cells were 4% formaldehyde fixed for 10 minutes and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab70475 at 10µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed used at a 1/1000 dilution for 1 hour at room temperature. ab195889 Anti-alpha Tubulin (Alexa Fluor® 594) was used as a counterstaining (pseudo-colored red) at a 1/250 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1 hour at room temperature.

ab70475 staining MUC1 in human breast cancer cell line T47D by Immunocytochemistry. Cells were fixed in methanol, blocked using 10% serum for 5 minutes at 25°C and then incubated with ab70475 at a 1/100 dilution for 1 hour at 25°C. The secondary used was an undiluted HRP conjugated goat anti-mouse polyclonal.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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