### Overview

**Product name**  
Anti-Cytokeratin 18 antibody [EPR17347]  

**Description**  
Rabbit monoclonal [EPR17347] to Cytokeratin 18  

**Host species**  
Rabbit  

**Tested applications**  
Suitable for: WB, ICC/IF, Flow Cyt, IHC-P  

**Species reactivity**  
Reacts with: Mouse, Rat, Human  

**Immunogen**  
Recombinant fragment within Mouse Cytokeratin 18 aa 200 to the C-terminus. The exact sequence is proprietary.  

Database link: P05784  

**Positive control**  

**General notes**  
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.

### Properties

**Form**  
Liquid  

**Storage instructions**  

**Storage buffer**  
Preservative: 0.01% Sodium azide  
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA  

**Purity**  
Protein A purified  

**Clonality**  
Monoclonal
Clone number: EPR17347
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab181597 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>
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<tbody>
<tr>
<td>WB</td>
<td>1/2000. Detects a band of approximately 47, 45 kDa (predicted molecular weight: 47 kDa).</td>
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<tr>
<td>ICC/IF</td>
<td>1/100.</td>
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<td>Flow Cyt</td>
<td>1/120.</td>
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<tr>
<td>IHC-P</td>
<td>1/800. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.</td>
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Target

Function: Involved in the uptake of thrombin-antithrombin complexes by hepatic cells (By similarity). When phosphorylated, plays a role in filament reorganization. Involved in the delivery of mutated CFTR to the plasma membrane. Together with KRT8, is involved in interleukin-6 (IL-6)-mediated barrier protection.

Tissue specificity: Expressed in colon, placenta, liver and very weakly in exocervix. Increased expression observed in lymph nodes of breast carcinoma.

Involvement in disease: Defects in KRT18 are a cause of cirrhosis (CIRRH) [MIM:215600].

Sequence similarities: Belongs to the intermediate filament family.

Post-translational modifications: Phosphorylation at Ser-34 increases during mitosis. Hyperphosphorylated at Ser-53 in diseased cirrhosis liver. Phosphorylation increases by IL-6. Proteolytically cleaved by caspases during epithelial cell apoptosis. Cleavage occurs at Asp-238 by either caspase-3, caspase-6 or caspase-7. O-glycosylated at multiple sites; glycans consist of single N-acetylglucosamine residues.

Cellular localization: Cytoplasm > perinuclear region.

Images
Immunofluorescent analysis of 4% Paraformaldehyde, 0.1% Triton X-100 permeabilized PC12 (Rat adrenal gland pheochromocytoma) cells, labeling Cytokeratin 18 with ab181597 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasm staining on PC-12 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
1. ab181597 at 1/100 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

Immunohistochemical analysis of paraffin-embedded Human colonic adenocarcinoma tissue labeling Cytokeratin 18 with ab181597 at 1/800 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. IHC showed membrane and cytoplasm staining on tumor cells of Human colon cancer. Counter stained with Hematoxylin.

Negative 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.
Flow cytometric analysis of 2% paraformaldehyde-fixed MCF7 (Human breast adenocarcinoma cell line) cells labeling Cytokeratin 18 with ab181597 at 1/120 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling Cytokeratin 18 with ab181597 at 1/800 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. IHC showed membrane and cytoplasm staining on hepatocytes of Human liver. Counter stained with Hematoxylin.

Negative 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.
Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Cytokeratin 18 with ab181597 at 1/800 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. IHC showed membrane and cytoplasm staining on hepatocytes of mouse liver. Counter stained with Hematoxylin.

Negative 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.

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling Cytokeratin 18 with ab181597 at 1/800 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. IHC showed membrane and cytoplasm staining on tubules of rat kidney. Counter stained with Hematoxylin.

Negative 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.
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized GR-M (Human Caucasian melanoma) cells, labeling Cytokeratin 18 with ab181597 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasm staining on GR-M cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
1. ab181597 at 1/100 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

Immunofluorescent analysis of 100% Methanol, 0.1% Triton X-100 permeabilized 4T-1 (Mouse mammary gland carcinoma cell line) cells, labeling Cytokeratin 18 with ab181597 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasm staining on 4T-1 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
1. ab181597 at 1/100 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.
**Western blot - Anti-Cytokeratin 18 antibody [EPR17347] (ab181597)**

**All lanes**: Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/10000 dilution

**Lane 1**: Mouse colon lysate  
**Lane 2**: Rat lung lysate  
**Lane 3**: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**  
**All lanes**: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size**: 47 kDa  
**Observed band size**: 45, 48 kDa  
*why is the actual band size different from the predicted?*

**Exposure time**: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.

The observed MW is consistent with what has been described in the literature (PMID:9298992).

**Western blot - Anti-Cytokeratin 18 antibody [EPR17347] (ab181597)**

**All lanes**: Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/5000 dilution

**Lane 1**: Mouse kidney lysate  
**Lane 2**: Mouse spleen lysate

Lysates/proteins at 10 µg per lane.

**Secondary**  
**All lanes**: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size**: 47 kDa  
**Observed band size**: 45, 48 kDa  
*why is the actual band size different from the predicted?*

**Exposure time**: 5 seconds
5% NFDM/TBST: Blocking and diluting buffer.

The observed MW is consistent with what has been described in the literature (PMID:9298992).

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Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/2000 dilution + F9 (Mouse embryo testicular cancer cell line) whole cell lysate at 10 µg

**Secondary**

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

**Predicted band size:** 47 kDa  
**Observed band size:** 45,48 kDa  
*why is the actual band size different from the predicted?*

**Exposure time:** 3 minutes

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5% NFDM/TBST: Blocking and diluting buffer.

The observed MW is consistent with what has been described in the literature (PMID:9298992).

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Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/10000 dilution + Human fetal skin lysate at 10 µg

**Secondary**

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

**Predicted band size:** 47 kDa  
**Observed band size:** 45,48 kDa  
*why is the actual band size different from the predicted?*

**Exposure time:** 2 minutes

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5% NFDM/TBST: Blocking and diluting buffer.

The observed MW is consistent with what has been described in the literature (PMID:9298992).
the literature (PMID:9298992).

**Western blot - Anti-Cytokeratin 18 antibody**

**All lanes**: Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/5000 dilution

**Lane 1**: Untreated HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

**Lane 2**: HeLa whole cell lysate treated with 1uM staurosporine for 4 hours

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size**: 47 kDa

**Observed band size**: 22, 45, 48 kDa

why is the actual band size different from the predicted?

**Exposure time**: 15 seconds

5% NFDM/TBST: Blocking and diluting buffer.

The 22 kDa cytokeratin 18 fragment was generated (lane 2) in the staurosporine-treated lysate that contained the active caspase 3.

**Western blot - Anti-Cytokeratin 18 antibody**

**All lanes**: Anti-Cytokeratin 18 antibody [EPR17347] (ab181597) at 1/5000 dilution

**Lane 1**: Untreated HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

**Lane 2**: HeLa whole cell lysate treated with 50uM Z-VAD-FMK for 4 hours

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size**: 47 kDa

**Observed band size**: 45, 48 kDa

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

**Exposure time:** 2 seconds

5% NFDM/TBST: Blocking and diluting buffer.

The generation of the 22kDa fragment was inhibited when the caspase 3 activity was blocked by the caspase inhibitor, Z-VAD-FMK.

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