Product Datasheet

Anti-Cytokeratin 18 antibody [C-04] ab668

Overview

Product name: Anti-Cytokeratin 18 antibody [C-04]
Description: Mouse monoclonal [C-04] to Cytokeratin 18
Host species: Mouse
Specificity: Human Cytokeratin

Tested applications: Suitable for: ICC, IHC-P, WB, Flow Cyt, IHC-Fr, IP, ICC/IF
Species reactivity: Reacts with: Mouse, Rat, Sheep, Goat, Horse, Hamster, Cow, Cat, Dog, Human, Pig, Common marmoset
Immunogen: Tissue, cells or virus corresponding to Cytokeratin 18. Cytoskeleton preparation of epidermal carcinoma cell line A431

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: pH: 7.40
Preservative: 0.097% Sodium azide
Constituent: PBS
Purity: Protein A purified
Purification notes: Purified from culture supernatant. Purity >95% by SDS-PAGE.
Clonality: Monoclonal
Clone number: C-04
Isotype: IgG1

Applications

Our Abpromise guarantee covers the use of ab668 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
### 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.

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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆</td>
<td>Use at an assay dependent concentration. Predicted molecular weight: 45 kDa.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 1μg for 10^6 cells. Also see PMID 18946470. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>1/600. PubMed: 23769181</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
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### Target

| Images | 6 | 2 |
Expression of epithelial and stem cell markers by DU145 colonies.

Expression of luminal (K18) and basal (K5) epithelial and stem cell markers (CD44, α2β1 integrin, Oct4 and BMI1) in DU145 colonies was determined by immunocytochemistry. (Panel A) Holo, mero and paraclone DU145 colonies were stained by immunocytochemistry with monoclonal antibodies against the target, detected with a FITC conjugated secondary antibody (Green) and counter stained with DAPI (blue).

Immunohistochemistry of mouse SG epithelial cells.

Immunohistochemical analysis of keratin 14, keratin 18 (ab668), p63, and alpha-smooth muscle actin (α-SMA) expression in normal SG tissue (SG) and P1 and P80 cell cultures. Scale bars = 10 μm.

ab668 staining Cytokeratin 18 in the human hepatocellular carcinoma cell line HepG2 by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with methanol/acetone (7:3) and blocked with 1% BSA for 1 hour at 20°C. Samples were incubated with primary antibody (1/100 in PBS) for 1 hour at 37°C. An Alexa Fluor® 488-conjugated rabbit anti-mouse IgG polyclonal was used as the secondary antibody at 1/100 dilution.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [C-04] (ab668)

ab668 (2 µg/ml) staining cytokeratin 18 in human skin tissue using an automated system (DAKO Autostainer Plus). Using this protocol there is strong cytoplasmic staining of sweat coils. Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.1 in a DAKO PT link. Slides were immersed in 3% H₂O₂/methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with hematoxylin and coverslipped under DePeX.

Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.

Overlay histogram showing HCT 116 (Human colorectal carcinoma cell line) cells stained with ab668 (red line).

The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Triton 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 (ab668, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HCT 116 cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Triton used under the same conditions.
ab668 staining Cytokeratin 18 in cat lung tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in citric acid. Samples were incubated with primary antibody (1/500 in TBS/BSA/azide) for 2 hours at 21°C. A biotin-conjugated goat anti-mouse IgG polyclonal (1/200) was used as the secondary antibody.

Coloured arrowheads indicate positivity. Good immunolabeling of bronchial tree epithelia (green), alveolar lining epithelium (red).

Goblet cells are negative (asterisk).

Heavily stained structures are submucosal mucous glands.

ab668 staining cytokeratin 18 in mouse epithelium tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/500 in TBS/BSA/azide) for 2 hours at 21°C. A biotin-conjugated Goat anti-mouse IgG polyclonal (1/200) was used as the secondary antibody.

Composite image of d14 embryo shows developing epithelia.

Upper image: positive simple epithelium of renal tubules

Lower image: negative stratified epithelium of epidermis

ab668 staining Cytokeratin 18 in horse primary bronchial epithelial cells by Immunocytochemistry/Immunofluorescence.

The cells were fixed in acetone and then blocked using 3% BSA for 10 hours at 4°C. Samples were then incubated with primary antibody at 1/100 for 1 hour at 22°C. The secondary antibody used was a goat anti-mouse conjugated to FITC used at a 1/200 dilution.
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