Product datasheet

Anti-Cytokeratin 18 antibody [LDK18] ab31844

Overview

Product name: Anti-Cytokeratin 18 antibody [LDK18]
Description: Mouse monoclonal [LDK18] to Cytokeratin 18
Host species: Mouse
Tested applications: Suitable for: Flow Cyt, ICC/IF, IHC-P
Species reactivity: Reacts with: Mouse, Human
Immunogen: Synthetic peptide: CSETN/TKVLHR-COOH, corresponding to C terminal amino acids 418-429 of Human Cytokeratin 18
Positive control: Simple epithelial cells This antibody gave a positive result when used in the following methanol fixed cell lines: HCT116.

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: Constituent: PBS
Purity: Protein A/G purified
Clonality: Monoclonal
Clone number: LDK18
Isotype: IgG1

Applications

Our Abpromise guarantee covers the use of ab31844 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.

Images:

ICC/IF image of ab31844 stained HCT116 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab31844 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (ab96879) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
Overlay histogram showing MCF7 cells stained with ab31844 (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 (ab31844, 0.1μg/1x10⁵ cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 1μg/1x10⁵ 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.

Ab31844 staining human normal colon tissue. Staining is localised to cytoplasm.
Left panel: with primary antibody at 2 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 buffer citrate pH 6.0 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.

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