# abcam

#### Product datasheet

## Anti-Cytokeratin 18 antibody [SP69] ab93741

Recombinant RabMAb

7 References 12 Images

Overview

**Product name** Anti-Cytokeratin 18 antibody [SP69]

**Description** Rabbit monoclonal [SP69] to Cytokeratin 18

**Host species** Rabbit

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

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide within Human Cytokeratin 18 aa 400 to the C-terminus (C terminal). The exact

> sequence is proprietary. Database link: P05783

Positive control IHC-P: Human prostate or breast carcinoma, Human stomach, and Human endometrium tissue;

Flow Cyt (Intra): HeLa cells; ICC/IF: HeLa cells. mIHC: Human breast.

**General notes** This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

**Properties** 

**Form** 

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.60

> Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

**Purity** Protein A/G purified

Purified from TCS by protein A/G. **Purification notes** 

Clonality Monoclonal

Clone number SP69 Isotype ΙgG

**Applications** 

#### The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab93741 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
mIHC		Use at an assay dependent concentration.
WB		1/200. Predicted molecular weight: 48 kDa.
IHC-P		1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Incubate for 30 minutes at RT. Perform heat mediated antigen retrieval before commencing with IHC staining protocol by boiling tissue sections in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at RT for 20 min.
ICC/IF		1/1000.
Flow Cyt (Intra)		1/2000.

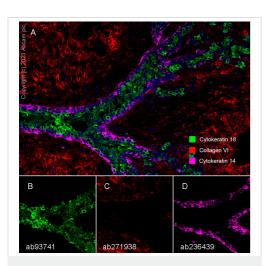
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П	<b>a</b>	ra	Δt

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.
Expressed in colon, placenta, liver and very weakly in exocervix. Increased expression observed in lymph nodes of breast carcinoma.
Defects in KRT18 are a cause of cirrhosis (CIRRH) [MIM:215600].
Belongs to the intermediate filament family.
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.

Cytoplasm > perinuclear region.

### **Images**

**Cellular localization** 



Multiplex immunohistochemistry - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast labelling Cytokeratin 18 with ab93741 at 1/200 dilution (1.02  $\mu$ g/mL) (B), Collagen VI with ab271938 at 1/500 dilution (2.084  $\mu$ g/ml) (C) and Cytokeratin 14 with ab236439 at 1/2000 dilution (0.519  $\mu$ g/ml) (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Panel A: merged staining of anti-Cytokeratin 14 (magenta; Opal™690), anti-Cytokeratin 18 (green; Opal™520) and anti-Collagen VI (red; Opal™570) on human breast.

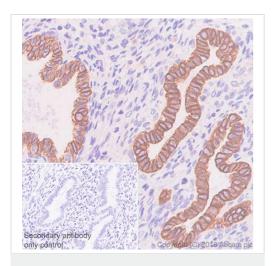
Panel B: anti-Cytokeratin 18 stained on luminal epithelial cells.

Panel C: anti-Collagen VI stained on stroma.

Panel D: anti-Cytokeratin 14 stained on myoepithelial cells.

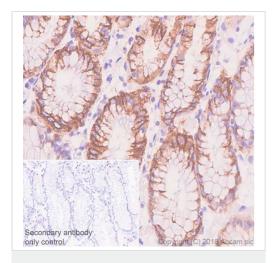
The section was incubated in three rounds of staining: in the order of <u>ab236439</u> for 30 mins, ab93741 for 10 mins, and <u>ab271938</u> for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.



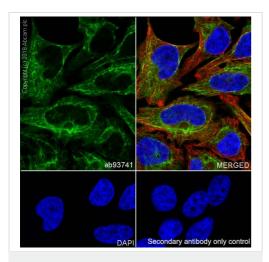
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human endometrium tissue sections labeling Cytokeratin 18 with ab93741 at 1/200 dilution (1.21 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



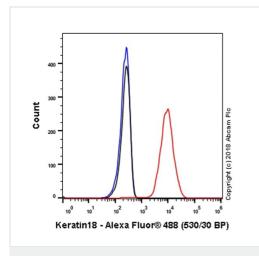
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach tissue sections labeling Cytokeratin 18 with ab93741 at 1/200 dilution (1.21 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



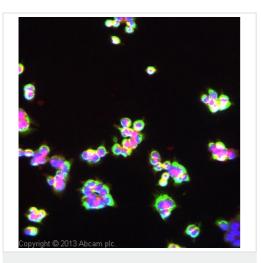
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Cytokeratin 18 with purified ab93741 at 1:100(2.4  $\mu$ g/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2  $\mu$ g/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

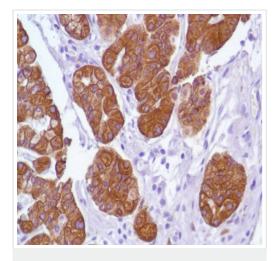
Flow Cytometry analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Cytokeratin 18 with purified ab93741 at 1:240 dilution (1.00 µg/ml) Red. Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1:2000 dilution. lsotype control - Rabbit monoclonal lgG (ab172730) / Black. Unlabeled control - Unlabelled cells / Blue.



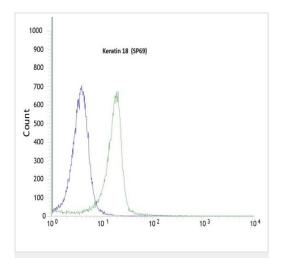
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

ICC/IF image of ab93741 stained HCT116 cells. The cells were 4% formaldehyde fixed (10 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 ab93741 at 1/500 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 Goat anti-Rabbit lgG (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.

Immunihistochemical staining of human stomach with ab93741.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

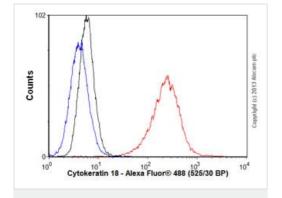


Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

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 (ab93741, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1µg/1x10<sup>6</sup> 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

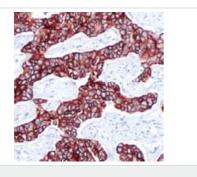
Overlay histogram showing MCF7 cells stained with ab93741 (red line). The cells were fixed with 80% methanol (5 min) and then



Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

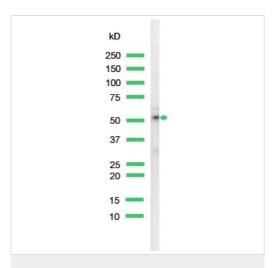
Flow cytometric analysis of rabbit anti-Cytokeratin 18 (SP69) antibody ab93741(1/100) in HeLa cells (green) compared to negative control of rabbit lgG (blue).

MCF7 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

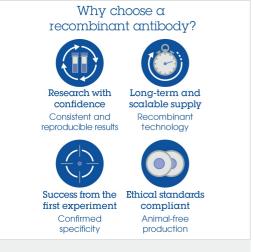
ab93741 at 1/200 dilution staining Cytokeratin 18 in formalin-fixed, paraffin-embedded Human breast carcinoma tissue.



Western blot - Anti-Cytokeratin 18 antibody [SP69] (ab93741)

Anti-Cytokeratin 18 antibody [SP69] (ab93741) at 1/25 dilution + A431 cell lysate

**Predicted band size:** 48 kDa **Observed band size:** 52 kDa



Anti-Cytokeratin 18 antibody [SP69] (ab93741)

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