abcam

Product datasheet

Anti-Cytokeratin 18 antibody [E431-1] ab32118

Recombinant RabMAb

6 Abreviews 32 References 17 Images

Overview

Product name	Anti-Cytokeratin 18 antibody [E431-1]
Description	Rabbit monoclonal [E431-1] to Cytokeratin 18
Host species	Rabbit
Specificity	Human Cytokeratin 18 (K18) was used as immunogen after isolation from cells pre-treated with okadaic acid or pervanadate to promote Tyr hyperphosphorylation.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Full length native protein (purified) corresponding to Human Cytokeratin 18.
Positive control	WB: A431 cell lysate. IHC-P: Human gastric adenocarcinoma, kidney, colon, breast carcinoma, brain, stomach and glioma tissue. ICC/IF: HT-29 cells; HeLa cells; (negative: U87-MG cells). Flow Cyt (intra): MCF7 cells.
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	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	E431-1
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32118 in the following tested applications.

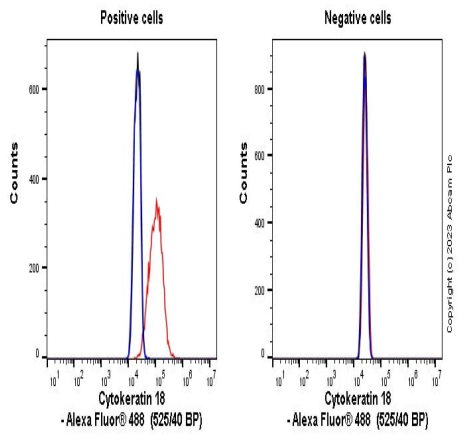
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/2000. Detects a band of approximately 48 kDa (predicted molecular weight: 48 kDa). For unpurified use at 1/10000
IHC-P	★★★★☆ (3)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★☆☆☆☆ (1)	1/100 - 1/500.

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



Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

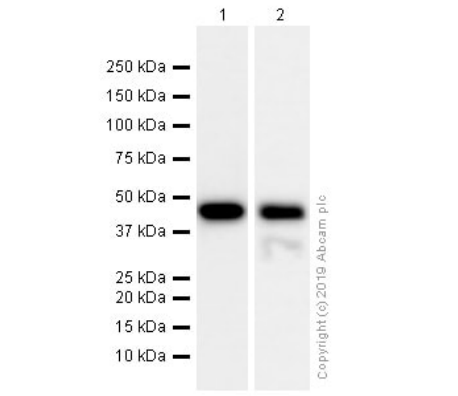
Flow cytometry overlay histogram showing left MCF7 positive cells and right negative A375 stained with ab32118 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32118) (1x 10^6 in 100µl at 0.008µg/ml (1/258750)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in MCF7 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Western blot - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

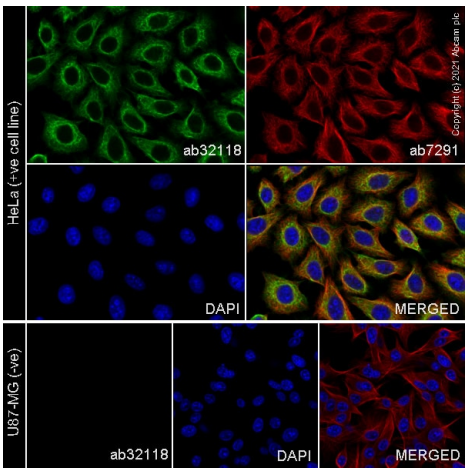
All lanes : Anti-Cytokeratin 18 antibody [E431-1] (ab32118) at 1/2000 dilution (Purified)

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 48 kDa
Observed band size: 48 kDa

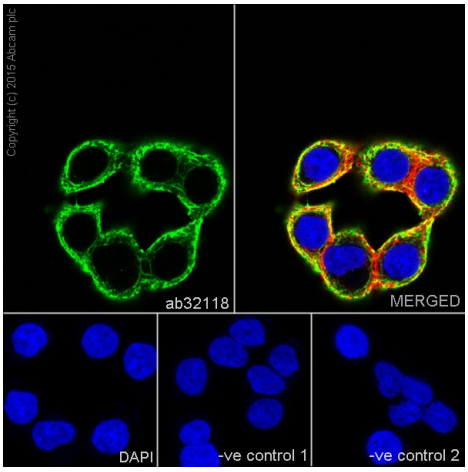


Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

Immunocytochemistry/Immunofluorescence analysis of HeLa (+ve) and U87-MG (-ve) cells labelling Cytokeratin 18 with ab32118 at 2 ug/ml overnight at +4°C. Cells were fixed with 100% Methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. A preadsorbed Alexa Fluor® 488-conjugated goat anti-rabbit IgG (**ab150081**) at 1/1000 dilution was used as the secondary antibody. The cells were co-stained with **ab7291**, a mouse anti-tubulin antibody (1/1000), using **ab150119**, a preadsorbed Alexa Fluor® 647-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 4% formaldehyde (10 min).

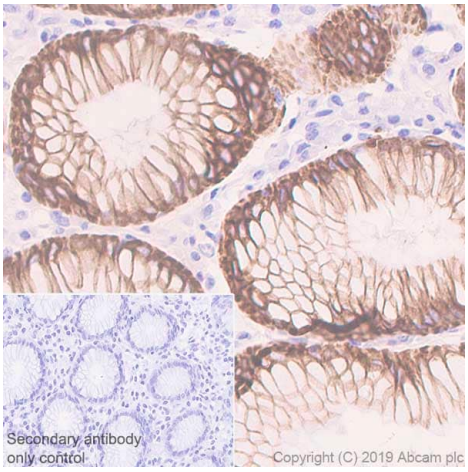


Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

Immunocytochemistry/Immunofluorescence analysis of HT-29 cells labelling Cytokeratin 18 with purified ab32118 at 1/500. Cells were fixed with 100% Methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (**ab150077**) at 1/1000 dilution was used as the secondary antibody. The cells were co-stained with **ab7291**, a mouse anti-tubulin antibody (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei were counterstained with DAPI (blue).

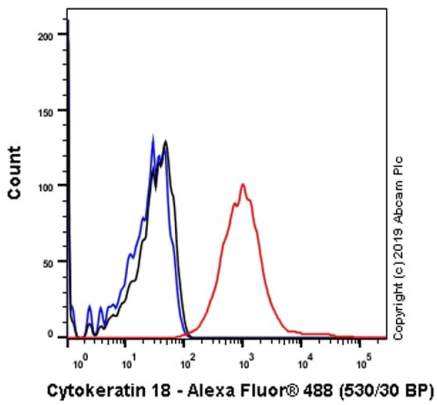
For negative control 1, rabbit primary antibody was used followed by anti-mouse secondary antibody (**ab150120**).
For negative control 2, mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**) were used.

Alexa Fluor® 488 (**ab194124**) conjugated version is available for this clone.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

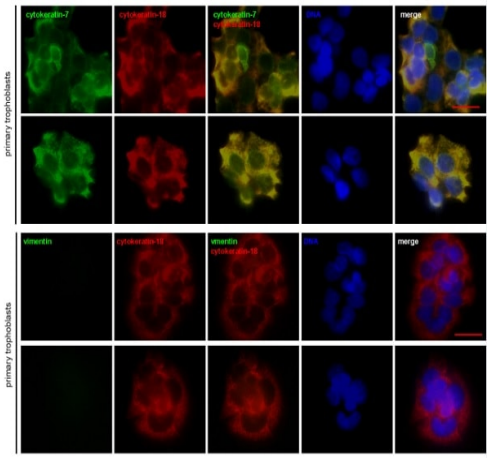
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach tissue sections labeling Cytokeratin 18 with purified ab32118 at 1/500 dilution (0.08 µg/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Cytokeratin 18 with purified ab32118 at 1/20 dilution (2µg/ml) (red). Cells were fixed with 80% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

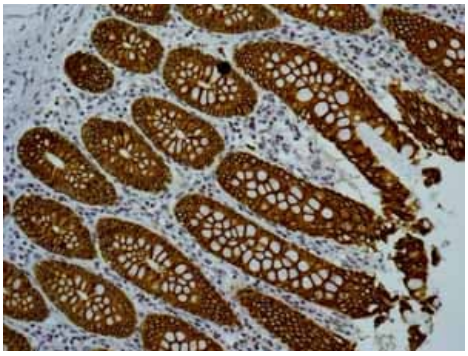
R-PE (**ab210410**) conjugated version is available for this clone.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

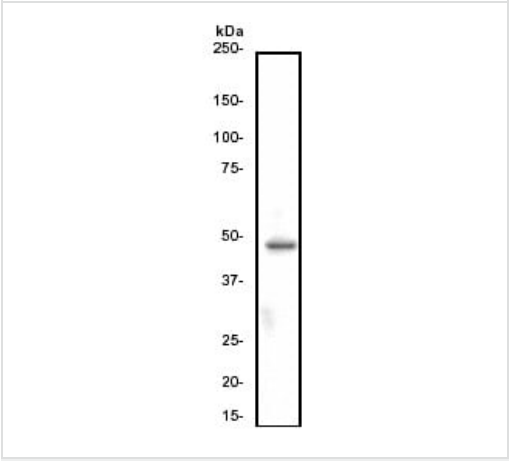
Indirect immunofluorescence staining with antibodies against DNA, cytokeratin-7 and cytokeratin-18 (upper panel) or against DNA, vimentin and cytokeratin-18 (lower panel). Representatives are presented. Scale bar: 20 µm.

Control or treated cells were fixed for 15 min with 4% PFA containing 0.1% Triton X-100 at room temperature. The following primary antibodies were used for staining: monoclonal mouse antibodies against vimentin and cytokeratin-7 (both 1:100, DAKO) and monoclonal rabbit antibody against cytokeratin-18 (1:50, Abcam). DNA was stained using DAPI (4',6-diamidino-2-phenylindole-dihydrochlorid) (Roche). Slides were examined using an Axio Imager 7.1 microscope (Zeiss) and images were taken using an Axio Cam MRm camera (Zeiss). The immunofluorescence stained slides were also examined by a confocal laser scanning microscope (CLSM) (Leica CTR 6500, Heidelberg). Images were processed using Photoshop.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

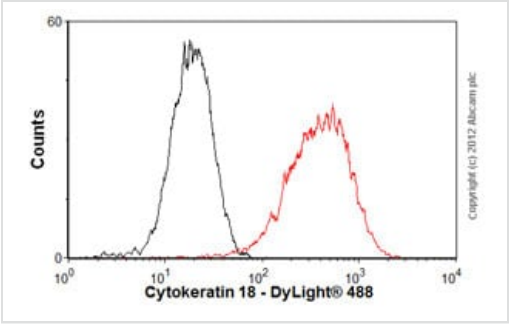
ab32118 showing positive staining in Normal colon tissue.



Western blot - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

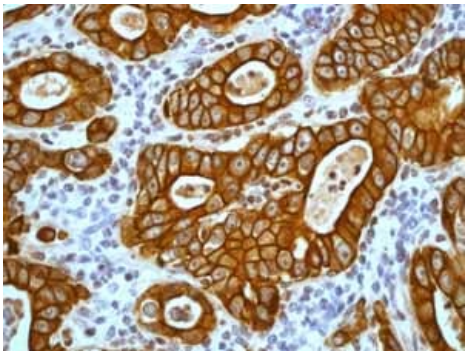
Anti-Cytokeratin 18 antibody [E431-1] (ab32118) at 1/10000 dilution + A431 cell lysate

Predicted band size: 48 kDa
Observed band size: 48 kDa



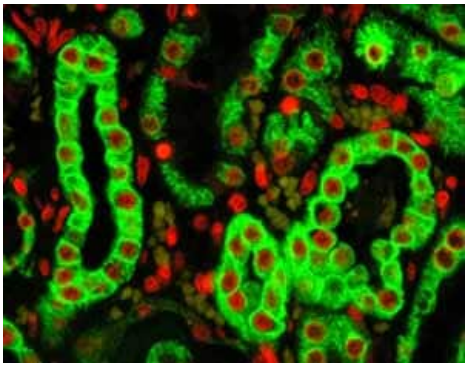
Flow Cytometry (Intracellular) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

Overlay histogram showing MCF7 cells stained with ab32118 (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 (ab32118, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. 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.



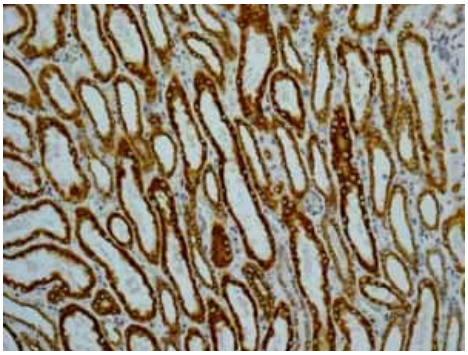
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

ab32118 showing positive staining in Gastric adenocarcinoma tissue.



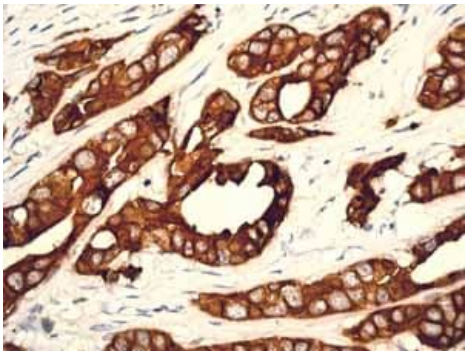
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

Fluorescent immunohistochemical analysis of paraffin-embedded human normal kidney tissue using ab32118. Green-CK18 red-PI



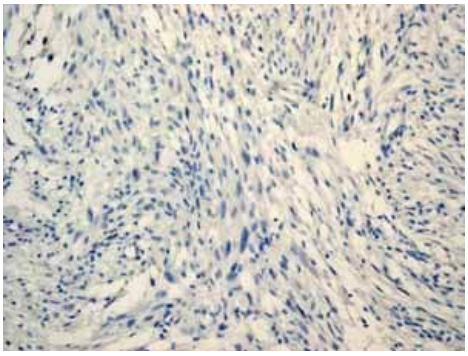
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

ab32118 showing positive staining in Normal kidney tissue.



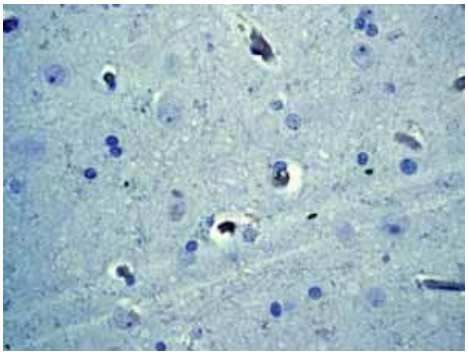
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

ab32118 showing positive staining in Breast carcinoma tissue.



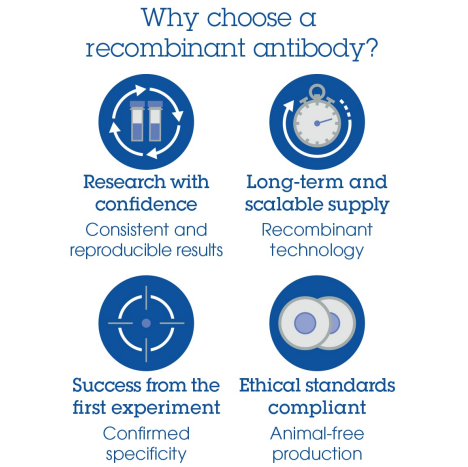
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

ab32118 showing negative staining in Glioma tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

ab32118 showing negative staining in Normal brain tissue.



Anti-Cytokeratin 18 antibody [E431-1] (ab32118)

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