abcam

Product datasheet

Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free ab219589

Recombinant RabMAb

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Overview

Product name Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free

Description Rabbit monoclonal [EPR1622Y] to Cytokeratin 20 - BSA and Azide free

Host species Rabbit

Specificity The immunogen of this antibody is 73% homolog with Mouse-Cytokeratin 20. This antibody gives

positive results for mouse samples in Western Blot only. Therefore we do not recommend this

antibody for mouse samples and do not cover mouse with our Abpromise guarantee.

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

Species reactivity Reacts with: Human

Predicted to work with: Rat, Goat, Pig, Common marmoset

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HT-29, DLD-1 and Human small intestine whole cell lysates. IHC-P: Human colon

adenocarcinoma and urinary bladder transitional carcinoma tissue. ICC: HT-29 cells. Flow Cyt

(intra): LoVo cells.

General notes ab219589 is the carrier-free version of ab76126.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

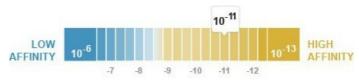
Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

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

Dissociation constant (K_D) $K_D = 3.10 \times 10^{-11} M$



Learn more about K_D

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR1622Y

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab219589 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)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 48 kDa.
IHC-P	**** (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function Plays a significant role in maintaining keratin filament organization in intestinal epithelia. When

phosphorylated, plays a role in the secretion of mucin in the small intestine.

Tissue specificity Expressed predominantly in the intestinal epithelium. Expressed in luminal cells of colonic

mucosa. Also expressed in the Merkel cells of keratinized oral mucosa; specifically at the tips of some rete ridges of the gingival mucosa, in the basal layer of the palatal mucosa and in the taste

buds of lingual mucosa.

Sequence similarities Belongs to the intermediate filament family.

Developmental stage First detected at embryonic week 8 in individual 'converted' simple epithelial cells of the

developing intestinal mucosa. In later fetal stages, synthesis extends over most goblet cells and a variable number of villus enterocytes. In the developing gastric and intestinal mucosa, expressed in all enterocytes and goblet cells as well as certain 'low-differentiated' columnar cells, whereas

the neuroendocrine and Paneth cells are negative.

Post-translational modifications

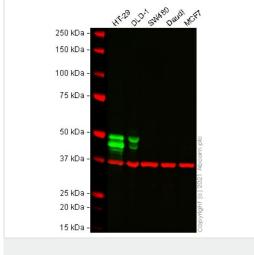
Hyperphosphorylation at Ser-13 occurs during the early stages of apoptosis but becomes less prominent during the later stages. Phosphorylation at Ser-13 also increases in response to stress

brought on by cell injury.

Proteolytically cleaved by caspases during apoptosis. Cleavage occurs at Asp-228.

Cellular localization Cytoplasm.

Images



Western blot - Anti-Cytokeratin 20 antibody

[EPR1622Y] - BSA and Azide free (ab219589)

All lanes : Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126) at 1/10000 dilution

Lane 1: HT-29 cell lysate

Lane 2: DLD-1 cell lysate

Lane 3: SW480 cell lysate

Lane 4 : Daudi cell lysate

Lane 5: MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 45-50 kDa

False colour image of Western blot: Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab76126 was shown to bind specifically to Cytokeratin 20. First, samples were run on an SDS-PAGE gel then

transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

<u>ab76126</u> at 1/100 dilution staining Cytokeratin 20 in human colon adenocarcinoma by Immunohistochemistry, Paraffin-embedded tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Immunocytochemistry/Immunofluorescence analysis of HT-29 (human colorectal adenocarcinoma) cells labelling Cytokeratin 20 with purified ab76126 at 1/500. Cells were fixed with 100% methanol. ab150077, Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

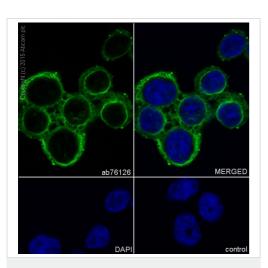
Secondary Only Control: PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

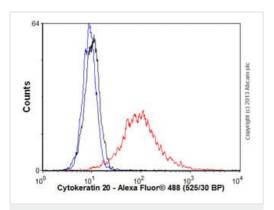


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - BSA and Azide free (ab219589)



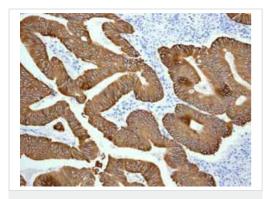
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free (ab219589)



Flow Cytometry (Intracellular) - Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free (ab219589)

Overlay histogram showing LoVo (Human colorectal adenocarcinoma cell line) cells stained with <u>ab76126</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 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 (<u>ab76126</u>, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor[®] 488 goat anti-rabbit lgG (H&L) (<u>ab150077</u>) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ 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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - BSA and Azide free (ab219589)

<u>ab76126</u> showing positive staining in human colonic adenocarcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



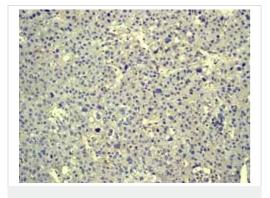
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - BSA and Azide free (ab219589)

<u>ab76126</u> showing positive staining in human urinary bladder transitional carcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

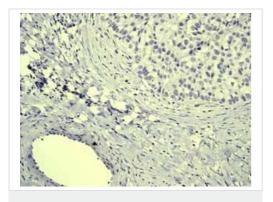


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free (ab219589)

<u>ab76126</u> showing **negative staining** in human hepatocellular carcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

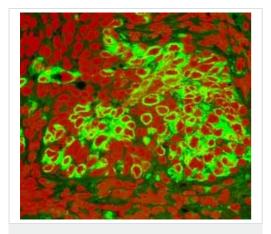


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody
[EPR1622Y] - BSA and Azide free (ab219589)

<u>ab76126</u> showing **negative staining** in human breast carcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

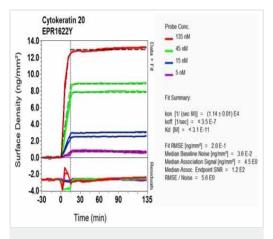


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody
[EPR1622Y] - BSA and Azide free (ab219589)

Fluorescent immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using <u>ab76126</u>. Green-CK20 red-PI

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

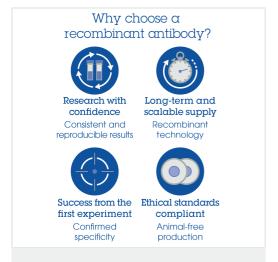


OI-RD Scanning - Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free (ab219589)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76126).



Anti-Cytokeratin 20 antibody [EPR1622Y] - BSA and Azide free (ab219589)

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