# abcam

## Product datasheet

## Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker ab76126

Recombinant RabMAb

★★★★★ 17 Abreviews 46 References 14 Images

#### Overview

**Product name** Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker

**Description** Rabbit monoclonal [EPR1622Y] to Cytokeratin 20 - Cytoskeleton Marker

**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

Reacts with: Human Species reactivity

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

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

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

and urinary bladder transitional carcinoma tissue. ICC: HT-29 cells. Flow Cyt (intra): LoVo 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**.

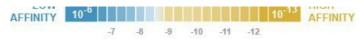
#### **Properties**

**Form** Liquid

Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. Storage instructions

 $K_D = 3.10 \times 10^{-11} M$ Dissociation constant (K<sub>D</sub>)

10-11



## Learn more about K<sub>D</sub>

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

supernatant

**Purity** Protein A purified

Clonality Monoclonal
Clone number EPR1622Y

**Isotype** IgG

## **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab76126 in the following tested applications.

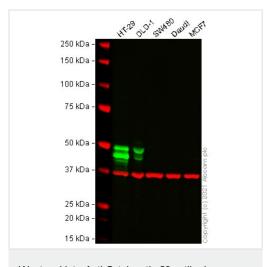
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	<b>★★★★ (3)</b>	1/100 - 1/500.
WB		1/10000 - 1/50000. Predicted molecular weight: 48 kDa.
IHC-P	<b>★★★★★</b> (12)	1/100 - 1/250. 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.

#### **Images**



Western blot - Anti-Cytokeratin 20 antibody

[EPR1622Y] - Cytoskeleton Marker (ab76126)

**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

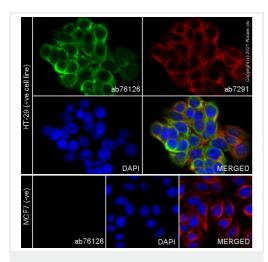
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

**Additional bands at:** 45 kDa. We are unsure as to the identity of these extra bands.

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<sup>®</sup> 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<sup>®</sup> 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.

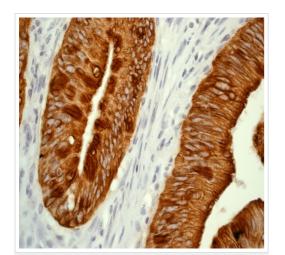


Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126)

ab76126 staining Cytokeratin 20 in HT-29 cells, with negative expression in MCF7 cells. The cells were fixed with 100% methanol (5 min), permeabilised 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. The cells were then incubated overnight at +4°C with ab76126 at 0.1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150119, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

This product also work with 4% formaldehyde (10 min) fixation under the same testing conditions.

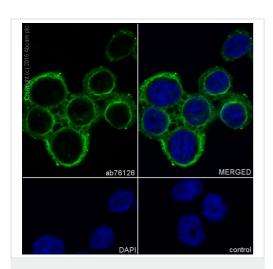


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - Cytoskeleton Marker (ab76126)

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

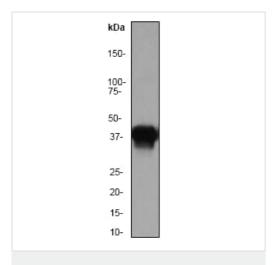
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 20 antibody [EPR1622Y] (ab76126)

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. <a href="mailto:ab150077">ab150077</a>, Alexa Fluor<sup>®</sup> 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.



Western blot - Anti-Cytokeratin 20 antibody [EPR1622Y] (ab76126)

Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126) at 1/50000 dilution + human small intestine lysate at 10  $\mu g$ 

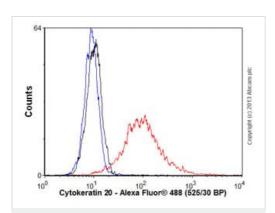
#### **Secondary**

goat anti-rabbit-HRP at 1/1000 dilution

**Predicted band size:** 48 kDa **Observed band size:** 44 kDa

Primary: ab76126, 1/50000 dilution

Sample: human small intestine lysate, 10 ug Secondary: goat anti-rabbit-HRP, 1/1000 dilution



Flow Cytometry (Intracellular) - Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126)

Overlay histogram showing LoVo (Human colorectal adenocarcinoma cell line) cells stained with ab76126 (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 (ab76126, 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) (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.



Western blot - Anti-Cytokeratin 20 antibody [EPR1622Y] (ab76126)

Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126) at 1/50000 dilution + Recombinant Human Cytokeratin 20 protein (ab73640) at 0.01 µg

#### **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

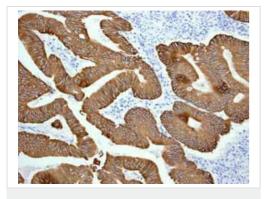
Predicted band size: 48 kDa

Exposure time: 20 seconds

Primary: ab76126, 1/50000 dilution

Sample: <u>ab73640</u>, 0.01 ug

#### Secondary: ab97080, 1/5000 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody
[EPR1622Y] - Cytoskeleton Marker (ab76126)

ab76126 showing positive staining in human colonic adenocarcinoma tissue.

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

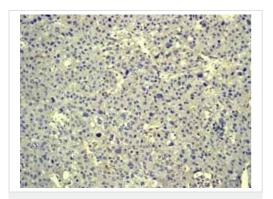


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - Cytoskeleton Marker (ab76126)

ab76126 showing positive staining in human urinary bladder transitional carcinoma tissue.

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

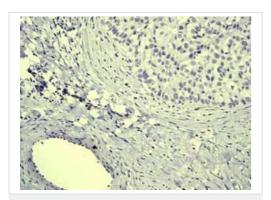


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - Cytoskeleton Marker (ab76126)

ab76126 showing **negative staining** in human hepatocellular carcinoma tissue.

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

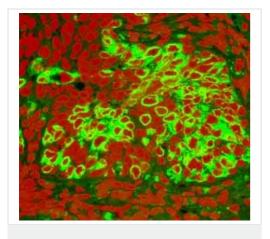


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 20 antibody

[EPR1622Y] - Cytoskeleton Marker (ab76126)

ab76126 showing **negative staining** in human breast carcinoma tissue.

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



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

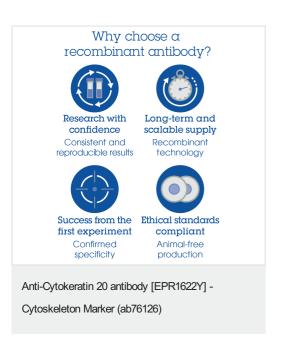
[EPR1622Y] - Cytoskeleton Marker (ab76126)

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

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

OI-RD Scanning - Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126) Equilibrium disassociation constant ( $K_D$ ) Learn more about  $K_D$ 

Click here to learn more about K<sub>D</sub>



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