abcam

ab9023

Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker

Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023) is a mouse monoclonal antibody detecting Cytokeratin 8 in **Western Blot, Flow Cytometry, IHC-P, IHC-Fr, ICC/IF**. Suitable for **Human, Rabbit, Rat**.

- KO validated for confirmed specificity
- Over 40 publications
- Trusted since 2003

Lab Essentials | KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.3 Preservative: 0.1% Sodium azide Constituents: PBS, 1% Fetal calf serum
Form	Liquid
Clonality	Monoclonal
Immunogen	Native Full Length Protein corresponding to Human KRT8. Database link P05787
Clone number	M20
Purity	Tissue culture supernatant

Reactivity data

ICC

Expected

Species	Rat, Human, Rabbit

Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	2 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Predicted

Species	Rat, Rabbit
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	-

Predicted

Species	Rat, Rabbit
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human

Dilution info	1/5 - 1/25
Notes	-

Predicted

Species	Rat, Rabbit
Dilution info	-
Notes	-

ICC/IF

Expected

Species	Rat, Human, Rabbit
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-Fr

Tested

Species	Human
Dilution info	1/100 - 1/200
Notes	For human colon 20min ice cold acetone fixation was carried out with 60min incubation with ab9023 at 37C.

Predicted

Species	Rat, Rabbit
Dilution info	-
Notes	-

Target data

See full target information KRT8

Function	Required for the formation of KRT8/KRT18 filaments that are involved in ARHGEF40-mediated actin stress fiber formation and tensional force-induced

stress fiber formation and reinforcement (PubMed:26823019). Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

What is this antibody validated in?
Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunohistochemistry (IHC-Fr), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Rabbit, Rat samples.

Trusted by the scientific community
Anti-Cytokeratin 8 [M20] - Cytoskeleton Marker (ab9023) was first used in a scientific publication in 2003 and has been cited over 40 times in peer-reviewed journals.

Reviewed by scientists
Anti-Cytokeratin 8 [M20] - Cytoskeleton Marker (ab9023) has over 10 independent reviews from customers.

Specificity confirmed
The specificity of Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023) has been confirmed by Western blot testing in Cytokeratin 8 Knockout HeLa cell line, ab255400.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

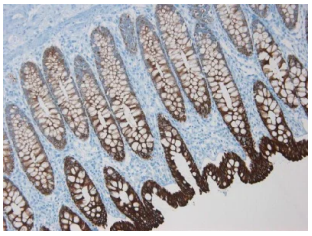
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

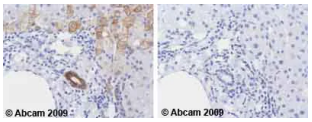
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9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

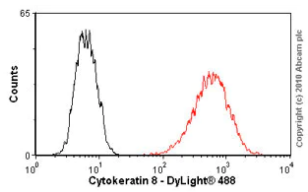
Immunohistochemistry on paraffin section of human colon



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Cytokeratin 8 antibody

ab9023 staining human normal liver parenchima tissue. Staining is localized to cell membrane. Left panel: with primary antibody at 4 ug/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 AR buffer EDTA pH 9.0 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.

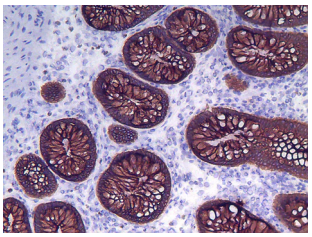


Flow Cytometry - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Flow Cytometry staining using mouse Anti-Cytokeratin 8 antibody

Overlay histogram showing MCF-7 cells stained with ab9023 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.5% 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 (ab9023, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H&L) (ab96879) at 1/250

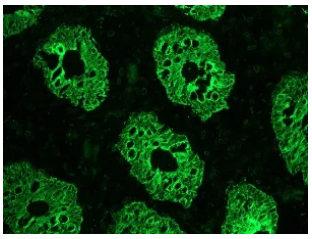
dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in MCF-7 cells fixed with methanol (5 min)/permeabilized in 0.5% PBS-Tween used under the same conditions.



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Immunohistochemistry (Frozen sections) staining using mouse Anti-Cytokeratin 8 antibody

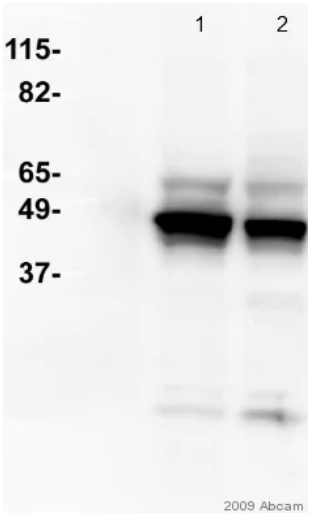
Immunohistochemistry analysis of frozen section of human colon labeling Cytokeratin 8 with ab9023.



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Immunohistochemistry (Frozen sections) staining using mouse Anti-Cytokeratin 8 antibody

Immunohistochemistry on frozen section of human colon



This image is a courtesy of Anonymous customer review

Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

All lanes:
Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023) at 1/2000 dilution

Lane 1:
Lysate prepared from human prostate cancer LNCaP cells at 50 µg

Lane 2:
Lysate prepared from human prostate cancer PC3 cells at 50 µg

Secondary

All lanes:
HRP-conjugated goat polyclonal to mouse IgG at 1/10000 dilution

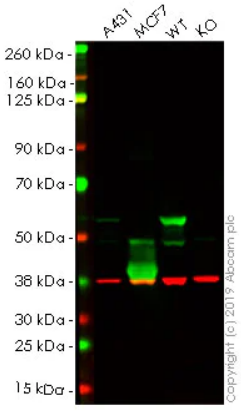
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 53 kDa

Observed band size: 26 kDa, 50 kDa, 65 kDa

Exposure time: 4min



Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Western blot staining using mouse Anti-Cytokeratin 8 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab9023 observed at 55 kDa. Red - loading control, ab181602 observed at 37 kDa.
ab9023 was shown to react with Cytokeratin 8 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255400 (knockout cell lysate ab263785) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. ab9023 and Anti-GAPDH antibody EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023) at 1/1000 dilution

Lane 1:
A431 cell lysate at 20 µg

Lane 2:
MCF7 cell lysate at 20 µg

Lane 3:
Wild-type HeLa cell lysate at 20 µg

Lane 4:
Western blot - Human KRT8 (Cytokeratin 8) knockout HeLa cell line (ab255400)

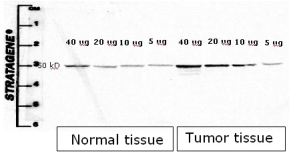
Lane 4:
Western blot - Human KRT8 (Cytokeratin 8) knockout HeLa cell lysate (ab263785)

Secondary

All lanes:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) at 1/20000 dilution

Predicted band size: 53 kDa

Observed band size: 37 kDa



Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

The image shows different concentration of total protein from normal and tumour colon tissues.

This picture was kindly supplied as part of the review submitted by Semona Rupchand.

The image shows different concentration of total protein from normal and tumour colon tissues. This picture was kindly supplied as part of the review submitted by Semona Rupchand.

All lanes:
Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Predicted band size: 53 kDa

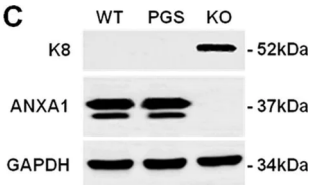


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Western blot - Anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker (ab9023)

Cytokeratin 8 Western blot staining using mouse Anti-Cytokeratin 8 antibody

Cytokeratin 8 western blot using anti-Cytokeratin 8 antibody [M20] - Cytoskeleton Marker ab9023. Publication image and figure legend from Belvedere, R., Bizzarro, V., et al., 2016, Sci Rep, PubMed 27412958.

ab9023 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab9023 please see the product overview.

(A) Cell surface expression of CD44 was analyzed by flow cytometry. The violet area in the plot is relative to human IgG1; CD44 signals are showed in green for WT MIA PaCa-2, in pink for PGS MIA PaCa-2 and in black for ANXA1 KO MIA PaCa-2. (B) RT-PCR for K18 mRNA expression measured on levels of HPRT. Values are expressed using the delta-delta Ct method to derive relative fold change. ***$p < 0.001$. (C,D) Western blots showing K8, ANXA1, lamin A/C, vimentin and GAPDH expression in WT, PGS and ANXA1 KO MIA PaCa-2 cells. (E,F) Vimentin and lamin A/C relative expression was analyzed by densitometry. The optical density of the protein bands was normalized on GAPDH levels giving to the control band an arbitrary value of 100. The blots were exposed to Las4000 (GE Healthcare Life Sciences) and the relative intensities of bands were determined using ImageQuant software (GE Healthcare Life Sciences). (G) Immunofluorescence analysis to detect ANXA1 (red; panels a, b, c), vimentin (yellow; panels d, e, f), lamin A/C (purple; panel g, h, i) and F-actin (green; panels l, m, n) in WT, PGS and ANXA1 KO MIA PaCa-2. Nuclei were stained with DAPI. Magnification 63 × 1.4 NA. Bar = 10 μm. (H) Fluorescence intensity for ANXA1, vimentin and lamin A/C signals (arbitrary units, A.U.) using ImageJ software; determined on 150 cells (for three independent experiments). The results relative to ANXA1 KO MIA PaCa-2 are representative ± SEM of almost three analyzed clones with a similar behaviour.

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