abcam

Product datasheet

Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker ab7754

KO VALIDATED

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Overview

Product name	Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker
Description	Mouse monoclonal [A53-B/A2] to Cytokeratin 19 - Cytoskeleton Marker
Host species	Mouse
Specificity	Rod domain of cytokeratin peptide 19 (40 kDa) in human tissue.
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to Human Cytokeratin 19. Human mammary carcinoma cell line MCF-7
Epitope	Rod domain of cytokeratin peptide 19.
Positive control	ICC/IF KO: MCF7 cells (MCF7-KRT19 KO used as a negative cell line). HepG2 cells. IHC-P: Human skin. Human liver tissue. WB: HT-29 lysate. Flow Cyt (Intra): MCF7 cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	A53-B/A2
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab7754 in the following tested applications.

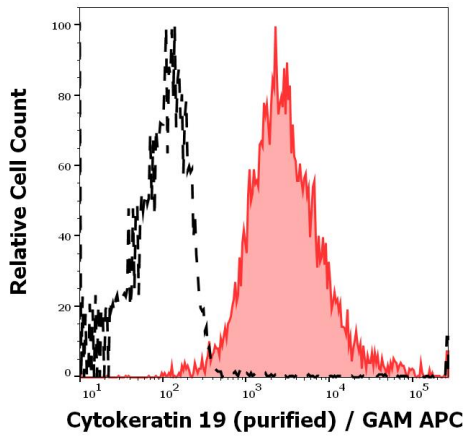
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (1)	Use at an assay dependent concentration. Signal can be observed in cells fixed with either methanol or paraformaldehyde.
WB	★★★★★ (3)	Use a concentration of 1 - 2 µg/ml. Predicted molecular weight: 44 kDa.
IHC-P	★★★★★ (1)	Use a concentration of 5 - 10 µg/ml. Perform enzymatic antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use a concentration of 1 - 5 µg/ml. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Target

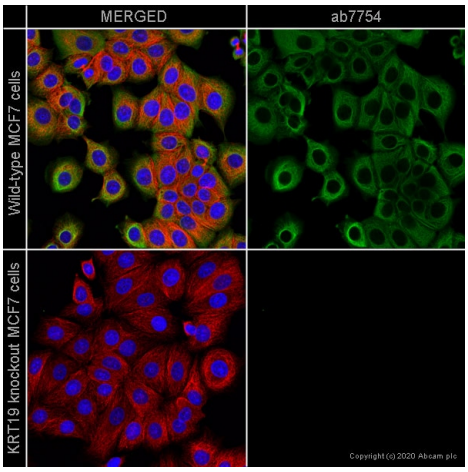
Function	Involved in the organization of myofibers. Together with KRT8, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Tissue specificity	Expressed in a defined zone of basal keratinocytes in the deep outer root sheath of hair follicles. Also observed in sweat gland and mammary gland ductal and secretory cells, bile ducts, gastrointestinal tract, bladder urothelium, oral epithelia, esophagus, ectocervical epithelium (at protein level). Expressed in epidermal basal cells, in nipple epidermis and a defined region of the hair follicle. Also seen in a subset of vascular wall cells in both the veins and artery of human umbilical cord, and in umbilical cord vascular smooth muscle. Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma in structures that contain dystrophin and spectrin.
Sequence similarities	Belongs to the intermediate filament family.
Developmental stage	Present in hair follicles at all stages of development.
Domain	This keratin differs from all other IF proteins in lacking the C-terminal tail domain.

Images



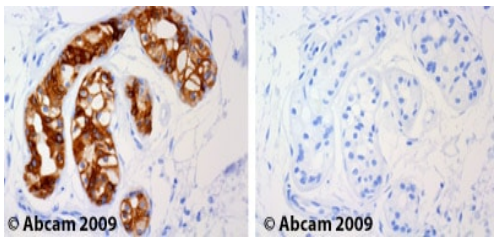
Flow Cytometry (Intracellular) - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

Separation of MCF-7 cells (red-filled) from human leukocytes (black-dashed) in flow cytometry analysis (intracellular staining) of peripheral whole blood spiked with MCF-7 cells stained using ab7754 (concentration in sample 3 µg/ml, GAM APC).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

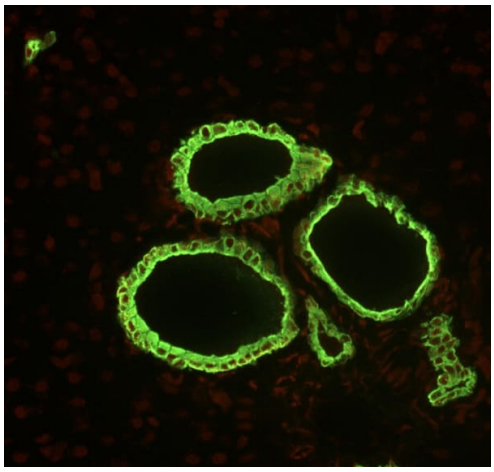
ab7754 staining Cytokeratin 19 in wild-type MCF7 cells (top panel) and KRT19 knockout MCF7 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab7754 at 1/500 dilution and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

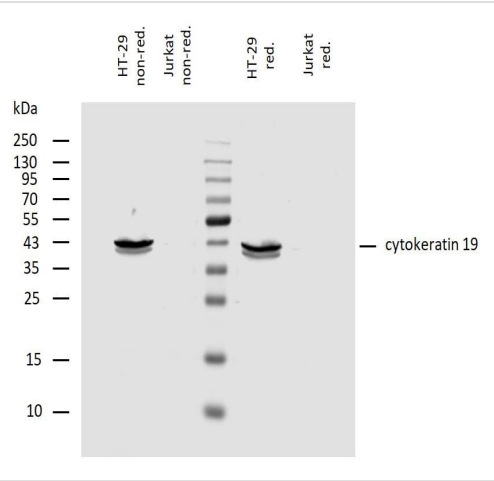
ab7754 staining Cytokeratin 19 in human skin.
Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.
Sections were stained using an automated system (DAKO Autostainer Plus), at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers citrate pH6.1. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

Immunohistochemical analysis of paraffin-embedded human liver tissue stained for Cytokeratin 19 using ab7754 at a 1/100 dilution followed by a GAM IgG-Alexa Fluor®488 diluted at 1/200 (green). Cell nuclei stained with PI (1 µg/ml; orange).

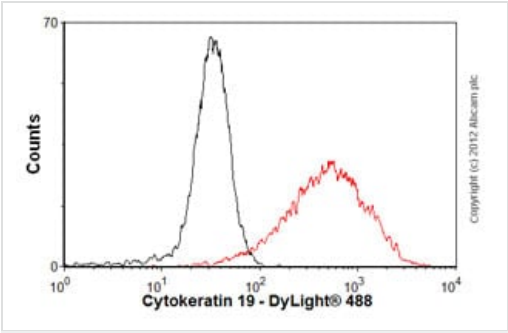


Western blot - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

Western blotting analysis of human cytokeratin 19 using ab7754 at 2 µg/ml on lysates of HT-29 (Human colorectal adenocarcinoma cell line) cell line and Jurkat (Human T cell leukemia cell line from peripheral blood) cell line (cytokeratin non-expressing cell line; negative control) under non-reducing and reducing conditions.

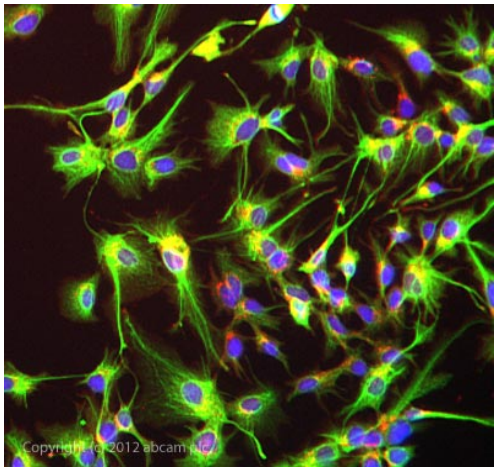
IRDye800-conjugated anti-mouse IgG1 secondary antibody.

A specific band was detected for cytokeratin 19 at approximately 40 kDa.



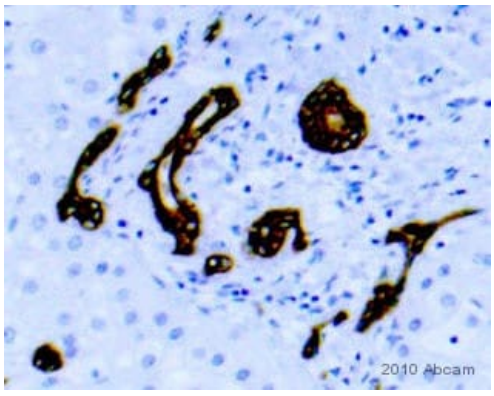
Flow Cytometry (Intracellular) - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

Overlay histogram showing MCF7 cells stained with ab7754 (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 (ab7754, 0.5µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

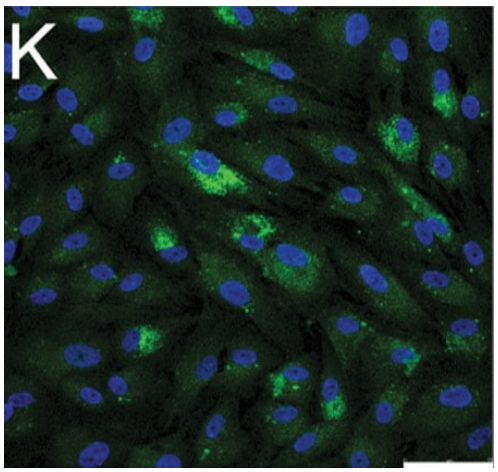
ICC/IF image of ab7754 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab7754 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (**ab96879**) IgG (H+L) used at a 1/1000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

This image is courtesy of an anonymous Abreview

ab7754 staining Cytokeratin 19 in Human liver tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 5% milk for 30 minutes at 37°C; antigen retrieval was by heat mediation in citrate buffer. Samples were incubated with primary antibody (1/1000 in antibody diluent) for 1 hour at 37°C. An undiluted HRP-conjugated goat anti-rabbit IgG polyclonal was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 19 antibody [A53-B/A2] - Cytoskeleton Marker (ab7754)

Image from Ferro F et al., PLoS One. 2012;7(7):e41774. Epub 2012 Jul 23. Fig 3.; doi:10.1371/journal.pone.0041774; July 23, 2012, PLoS ONE 7(7): e41774.

Immunofluorescence analysis of 1 month hepato-differentiated Human dental pulp stem cells, staining Cytokeratin 19 with ab7754 at 1/60 dilution. A FITC-conjugated anti-mouse IgG was used as the secondary antibody.

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