abcam

Product datasheet

Anti-Cytokeratin 19 antibody [BA-17] ab7755

KO VALIDATED

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Overview

Product name	Anti-Cytokeratin 19 antibody [BA-17]
Description	Mouse monoclonal [BA-17] to Cytokeratin 19
Host species	Mouse
Specificity	Cytokeratin peptide 19 (40 kDa) in human tissue.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to Human Cytokeratin 19. Mammary organoids Database link: **P08727**
Positive control	ICC/IF KO: HepG2, MCF7 cells (MCF7-KRT19 KO used as a negative cell line). WB: MCF-7, HepG2, SW480, MDA-MB-361 cell lysates. IHC-P: Human skin. IP: HepG2 cell extract. 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	BA-17
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab7755 in the following tested applications.

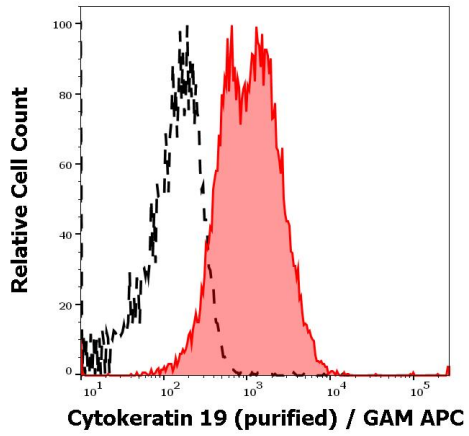
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use a concentration of 1 - 5 µg/ml. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB	★★★★★ (2)	Use a concentration of 1 - 2 µg/ml.
IHC-P	★★★★☆ (2)	Use a concentration of 5 - 10 µg/ml. Perform enzymatic antigen retrieval before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

Target

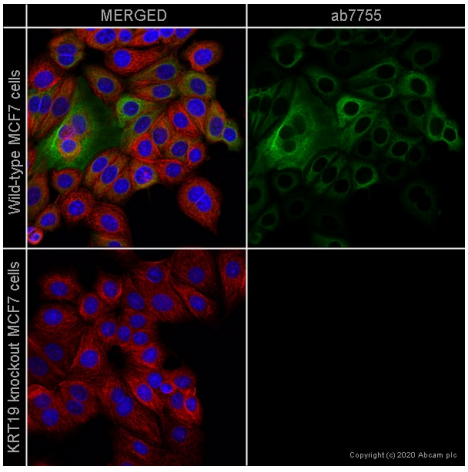
Function	Involved in the organization of myofibers. Together with KRT8, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Tissue specificity	Expressed in a defined zone of basal keratinocytes in the deep outer root sheath of hair follicles. Also observed in sweat 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



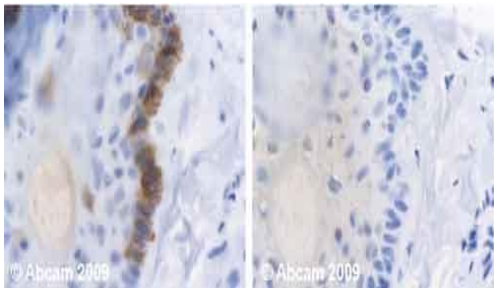
Flow Cytometry (Intracellular) - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

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 ab7755 (concentration in sample 3 µg/ml, GAM APC).



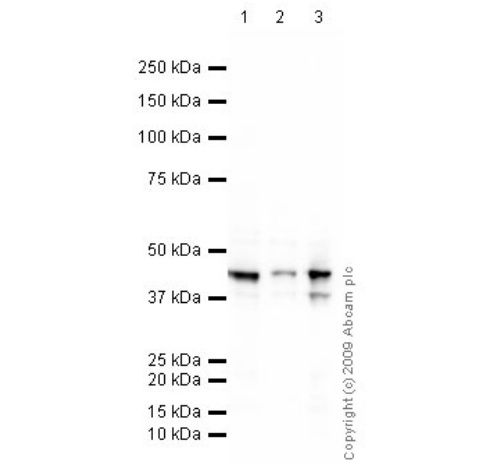
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

ab7755 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 ab7755 at 5µg/ml concentration 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 [BA-17] (ab7755)

Human normal skin. Staining is observed in the cytoplasm (epidermal basal cells). 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 EDTA pH 9.0 in a DAKO PT Link. 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 mouse 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.



Western blot - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

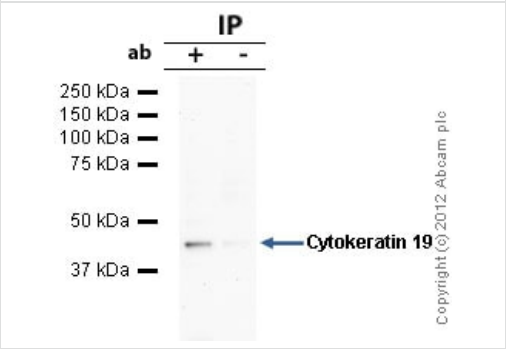
All lanes : Anti-Cytokeratin 19 antibody [BA-17] (ab7755) at 5 µg/ml

Lane 1 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 2 : SW480 (Human colon adenocarcinoma cell line) Whole Cell Lysate
Lane 3 : MDA-MB-361 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

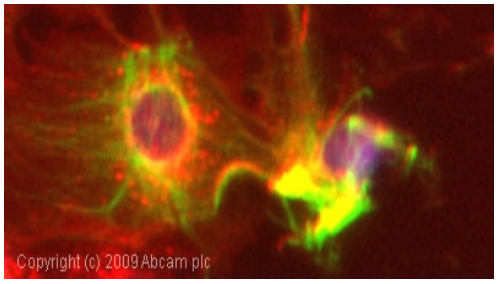
Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Observed band size: 44 kDa



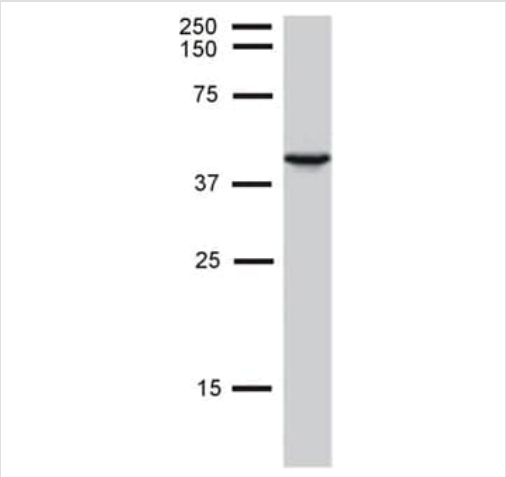
Immunoprecipitation - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

Cytokeratin 19 was immunoprecipitated using 0.5mg HepG2 whole cell extract, 5µg of Mouse monoclonal to Cytokeratin 19 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, HepG2 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab7755.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 44kDa: Cytokeratin 19



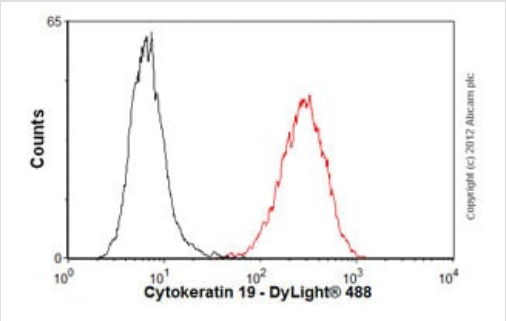
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

ICC/IF image of ab7755 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 (ab7755, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse 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.



Western blot - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

Anti-Cytokeratin 19 antibody [BA-17] (ab7755) + Cell lysates prepared from human MCF-7 cells



Flow Cytometry (Intracellular) - Anti-Cytokeratin 19 antibody [BA-17] (ab7755)

Overlay histogram showing MCF7 cells stained with ab7755 (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 (ab7755, 1µg/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 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 MCF7 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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