abcam

Product datasheet

Anti-Cytokeratin 14 antibody [SP53] ab119695

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Cytokeratin 14 antibody [SP53]
Description	Rabbit monoclonal [SP53] to Cytokeratin 14
Host species	Rabbit
Tested applications	**Suitable for:** mIHC, ICC/IF, IHC-P, WB, Flow Cyt (Intra), IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Cow, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431 cell lysate and human skin tissue lysate. IHC-P: Human prostate tissue. IHC-Fr: Mouse and Rat skin tissue. ICC/IF: A431 cells. Flow Cyt: A431 cells. mIHC: Human breast.
General notes	**This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A/G purified
Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP53
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab119695 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
mIHC		Use at an assay dependent concentration.
ICC/IF	★★★★★ (1)	Use a concentration of 5 µg/ml.
IHC-P	★★★★★ (1)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/25. Detects a band of approximately 50 kDa (predicted molecular weight: 52 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr	★★★★★ (1)	Use at an assay dependent concentration.

Target

Function

The nonhelical tail domain is involved in promoting KRT5-KRT14 filaments to self-organize into large bundles and enhances the mechanical properties involved in resilience of keratin intermediate filaments in vitro.

Tissue specificity

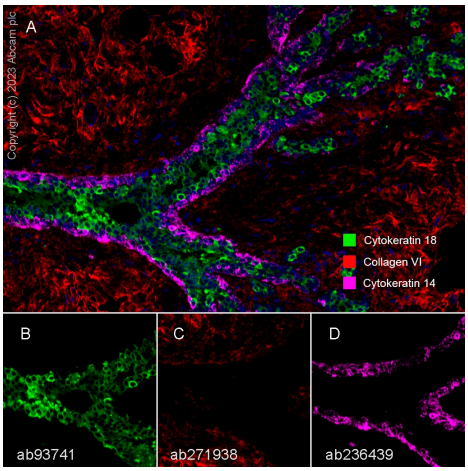
Detected in the basal layer, lowered within the more apically located layers specifically in the stratum spinosum, stratum granulosum but is not detected in stratum corneum. Strongly expressed in the outer root sheath of anagen follicles but not in the germinative matrix, inner root sheath or hair. Found in keratinocytes surrounding the club hair during telogen.

Involvement in disease

Defects in KRT14 are a cause of epidermolysis bullosa simplex Dowling-Meara type (DM-EBS) [MIM:131760]. DM-EBS is a severe form of intraepidermal epidermolysis bullosa characterized by generalized herpetiform blistering, milia formation, dystrophic nails, and mucous membrane involvement.
Defects in KRT14 are a cause of epidermolysis bullosa simplex Weber-Cockayne type (WC-EBS) [MIM:131800]. WC-EBS is a form of intraepidermal epidermolysis bullosa characterized by blistering limited to palmar and plantar areas of the skin.
Defects in KRT14 are a cause of epidermolysis bullosa simplex Koebner type (K-EBS) [MIM:131900]. K-EBS is a form of intraepidermal epidermolysis bullosa characterized by generalized skin blistering. The phenotype is not fundamentally distinct from the Dowling-Meara type, although it is less severe.
Defects in KRT14 are the cause of epidermolysis bullosa simplex autosomal recessive (AREBS) [MIM:601001]. AREBS is an intraepidermal epidermolysis bullosa characterized by localized blistering on the dorsal, lateral and plantar surfaces of the feet.
Defects in KRT14 are the cause of Naegeli-Franceschetti-Jadassohn syndrome (NFJS) [MIM:161000]; also known as Naegeli syndrome. NFJS is a rare autosomal dominant form of ectodermal dysplasia. The cardinal features are absence of dermatoglyphics (fingerprints), reticular cutaneous hyperpigmentation (starting at about the age of 2 years without a preceding inflammatory stage), palmoplantar keratoderma, hypohidrosis with diminished sweat gland function and discomfort provoked by heat, nail dystrophy, and tooth enamel defects.
Defects in KRT14 are the cause of dermatopathia pigmentosa reticularis (DPR) [MIM:125595].

	DPR is a rare ectodermal dysplasia characterized by lifelong persistent reticulate hyperpigmentation, noncicatricial alopecia, and nail dystrophy.
Sequence similarities	Belongs to the intermediate filament family.
Cellular localization	Cytoplasm. Nucleus. Expressed in both as a filamentous pattern.

Images



Multiplex immunohistochemistry - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

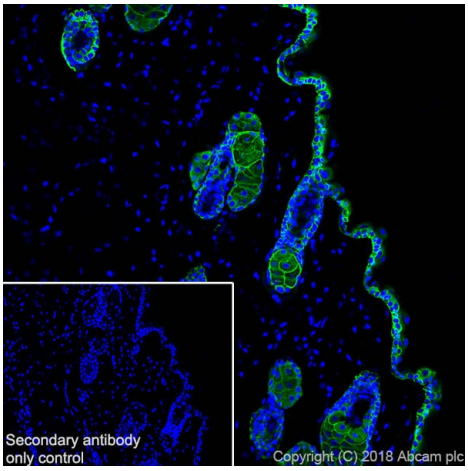
This data was developed using the same antibody clone in a different buffer formulation (**ab236439**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast labelling Cytokeratin 18 with **ab93741** at 1/200 dilution (1.02 µg/mL) (B), Collagen VI with **ab271938** at 1/500 dilution (2.084 µg/ml) (C) and Cytokeratin 14 with **ab236439** at 1/2000 dilution (0.519 µg/ml) (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Panel A: merged staining of anti-Cytokeratin 14 (magenta; Opal™690), anti-Cytokeratin 18 (green; Opal™520) and anti-Collagen VI (red; Opal™570) on human breast.
Panel B: anti-Cytokeratin 18 stained on luminal epithelial cells.
Panel C: anti-Collagen VI stained on stroma.
Panel D: anti-Cytokeratin 14 stained on myoepithelial cells.

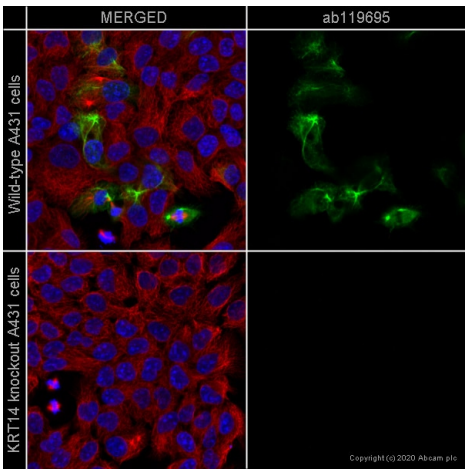
The section was incubated in three rounds of staining: in the order of **ab236439** for 30 mins, **ab93741** for 10 mins, and **ab271938** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



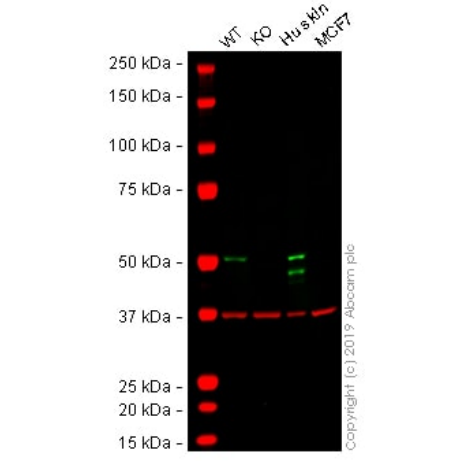
Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

Immunohistochemistry (Frozen) analysis of mouse skin tissue section labeling Cytokeratin 14 with purified ab119695. Sections were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Antigen retrieval was heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

ab119695 staining KRT14 in wild-type A431 cells (top panel) and KRT14 knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then 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 ab119695 at 5µg/ml concentration and **ab7291** (Mouse monoclonal to alpha 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 rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) 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).



Western blot - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

All lanes : Anti-Cytokeratin 14 antibody [SP53] (ab119695) at 1/93 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : KRT14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : Human skin whole tissue lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

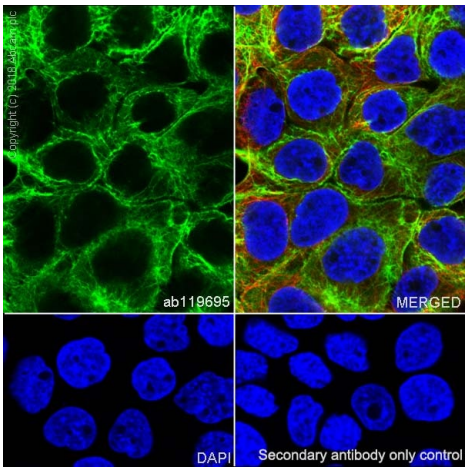
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 52 kDa

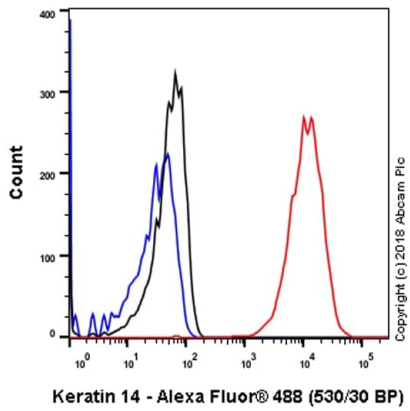
Lanes 1 - 4: Merged signal (red and green). Green - ab119695 observed at 52 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab119695 was shown to react with KRT14 in A431 wild-type cells in Western blot. Loss of signal was observed when KRT14 knockout sample was used. A431 wild-type and KRT14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab119695 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 93 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



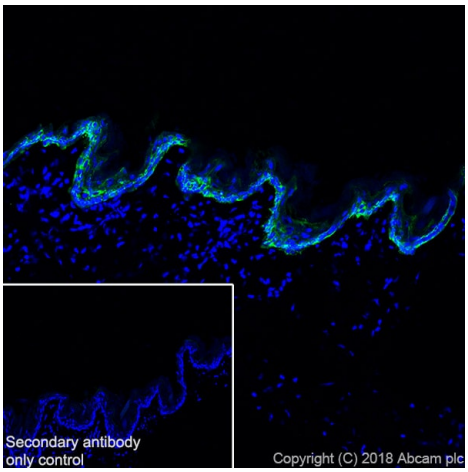
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

Immunocytochemistry/ Immunofluorescence analysis of A431(human epidermoid carcinoma epithelial cell) cells labeling Cytokeratin 14 with purified ab119695. Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



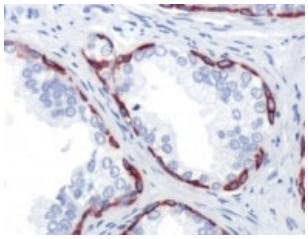
Flow Cytometry (Intracellular) - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

Flow cytometry analysis of A431 (human epidermoid carcinoma) labeling Cytokeratin 14 with purified ab119695 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as a secondary antibody. Isotype control -Rabbit monoclonal IgG (**ab172730**) (Black). Unlableled control -Unlabelled cells (blue).



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

Immunohistochemistry (Frozen) analysis of rat skin tissue section labeling Cytokeratin 14 with purified ab119695. Sections were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Antigen retrieval was heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 14 antibody [SP53] (ab119695)

Human prostate tissue stained with ab119695 at a dilution of 1/100.

Anti-Cytokeratin 14 antibody [SP53] (ab119695)

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