abcam

Product datasheet

Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free ab236439





RabMAb

1 References 9 Images

Overview

Product name Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free

Description Rabbit monoclonal [SP53] to Cytokeratin 14 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, Flow Cyt (Intra), ICC/IF, IHC-Fr, mIHC

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Pig

Immunogen Synthetic peptide within Human Cytokeratin 14 (C terminal). The exact sequence is proprietary.

Database link: P02533

Positive control WB: A431 cell lysate and human skin tissue lysate. IHC-P: Human prostate tissues. IHC-Fr:

Mouse and Rat skin tissue. ICC/IF: A431 cells. Flow Cyt (intra): A431 cells. mIHC: Human breast.

General notes ab236439 is the carrier-free version of <u>ab119695</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

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This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A/G purified

Purification notes Purified from TCS by protein A/G.

Clonality Monoclonal

Clone number SP53 lsotype lgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab236439 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 IHC-P Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. **WB** Use at an assay dependent concentration. 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. ICC/IF Use a concentration of 5 µg/ml. IHC-Fr Use at an assay dependent concentration.

Target

mIHC

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

Use at an assay dependent concentration.

intermediate filaments in vitro.

Tissue specificity Detected in the basal layer, lowered within the more apically located layers specifically in the

Involvement in disease

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.

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.

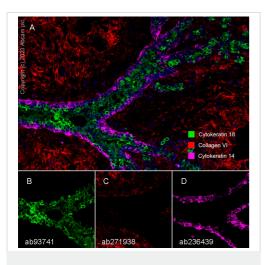
Sequence similarities

Cellular localization

Belongs to the intermediate filament family.

Cytoplasm. Nucleus. Expressed in both as a filamentous pattern.

Images



Multiplex immunohistochemistry - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast labelling Cytokeratin 18 with <u>ab93741</u> at 1/200 dilution (1.02 μ g/mL) (B), Collagen VI with <u>ab271938</u> at 1/500 dilution (2.084 μ g/ml) (C) and Cytokeratin 14 with ab236439 at 1/2000 dilution (0.519 μ g/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, <u>ab93741</u> for 10 mins, and <u>ab271938</u> for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

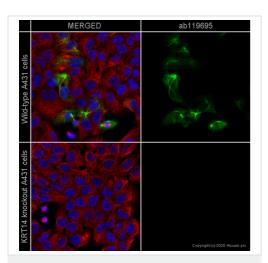
The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.

Secondary antibody only control Copyright (C) 2018 Alscam plc

Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

Immunohistochemistry (Frozen) analysis of mouse skin tissue section labeling Cytokeratin 14 with purified <u>ab119695</u> at 1/500 (3.8 µg/ml). 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 lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

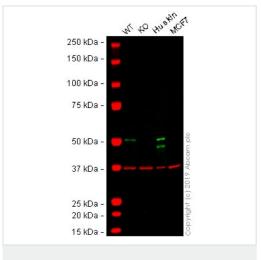
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab119695</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab119695</u>). <u>ab119695</u> 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 <u>ab119695</u> at 5μg/ml concentration and <u>ab7291</u> (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 lgG (Alexa Fluor[®] 488) (<u>ab150081</u>) at 2 μg/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor[®] 594) (<u>ab150120</u>) 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] - BSA and Azide free (ab236439)

All lanes : Anti-Cytokeratin 14 antibody [SP53] (<u>ab119695</u>) 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.

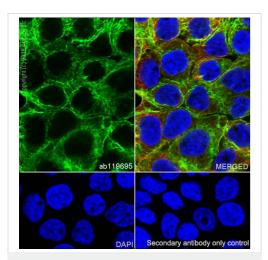
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab119695**).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab119695</u> observed at 52 kDa. Red - loading control, <u>ab8245</u> (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.

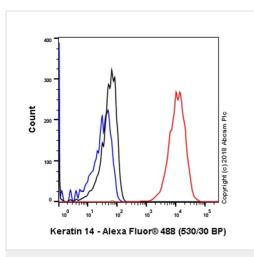


Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

(human epidermoid carcinoma epithelial cell) cells labeling
Cytokeratin 14 with purified <u>ab119695</u> at 1/2000 (0.95 μg/ml). Cells
were fixed in 4% paraformaldehyde and permeabilized with 0.1%
Triton X-100. Cells were counterstained with <u>ab195889</u> Anti-alpha
Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)
1/200 (2.5 μg/ml). Goat anti rabbit lgG (Alexa Fluor[®] 488,
<u>ab150077</u>) was used as the secondary antibody at 1/1000 (2
μg/ml) dilution. DAPI was used as nuclear counterstain. PBS
instead of the primary antibody was used as the secondary
antibody only control.
This data was developed using the same antibody clone in a

Immunocytochemistry/ Immunofluorescence analysis of A431

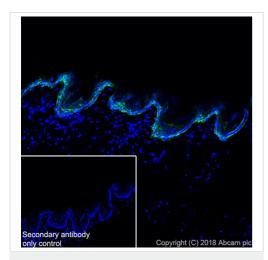
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab119695).



Flow Cytometry (Intracellular) - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

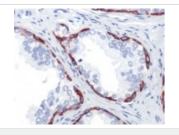
Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) labeling Cytokeratin 14 with purified <u>ab119695</u> at 1/1900 dilution (1.01µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) at 1/2000 dilution was used as a secondary antibody. Isotypecontrol - Rabbit monoclonal lgG (<u>ab172730</u>) (black). Unlableed control - Unlabelled cells (blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab119695</u>).



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439) Immunohistochemistry (Frozen) analysis of rat skin tissue section labeling Cytokeratin 14 with purified <u>ab119695</u> at 1/500 (3.8 μg/ml). 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 lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 μg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

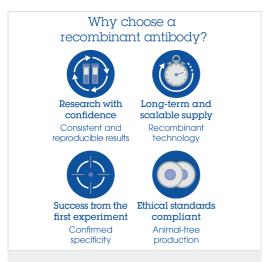
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab119695).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

Formalin-fixed, Paraffin-embedded Human prostate tissue stained for Cytokeratin 14 uisng <u>ab119700</u> in Immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab119700).



Anti-Cytokeratin 14 antibody [SP53] - BSA and Azide free (ab236439)

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