# abcam

# Product datasheet

# Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free ab214586



\*\*\*\* 2 Abreviews 9 References 21 Images

#### Overview

Product name Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free

**Description** Rabbit monoclonal [EP1601Y] to Cytokeratin 5 - BSA and Azide free

Host species Rabbit

Specificity Mouse reactivity is based on IHC (positive tissues: Liver, lung, brain and skin). However, WB was

negative for Mouse brain, heart, kidney and spleen. There is background staining in mouse and

rat islet.

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

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control A431 cells. Human transitional urinary bladder carcinoma. IHC-P: human normal skin tissue.

**General notes** ab214586 is the carrier-free version of <u>ab52635</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 <u>conjugation kits</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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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Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

# **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 purified

Clonality Monoclonal
Clone number EP1601Y

**Isotype** IgG

#### **Applications**

# The Abpromise guarantee

Our Abpromise guarantee covers the use of ab214586 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 at an assay dependent concentration.  ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P	**** (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 62 kDa (predicted molecular weight: 62 kDa).
ICC/IF		Use at an assay dependent concentration.

# **Target**

#### Involvement in disease

Defects in KRT5 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 KRT5 are the cause of epidermolysis bullosa simplex with migratory circinate erythema (EBSMCE) [MIM:609352]. EBSMCE is a form of intraepidermal epidermolysis bullosa

characterized by unusual migratory circinate erythema. Skin lesions appear from birth primarily on the hands, feet, and legs but spare nails, ocular epithelia and mucosae. Lesions heal with brown pigmentation but no scarring. Electron microscopy findings are distinct from those seen in the DM-EBS, with no evidence of tonofilament clumping.

Defects in KRT5 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 KRT5 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, althought it is less severe.

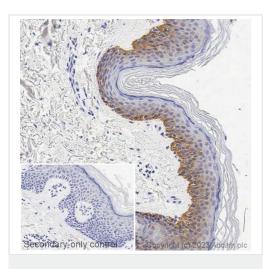
Defects in KRT5 are the cause of epidermolysis bullosa simplex with mottled pigmentation (MP-EBS) [MIM:131960]. MP-EBS is a form of intraepidermal epidermolysis bullosa characterized by blistering at acral sites and 'mottled' pigmentation of the trunk and proximal extremities with hyperand hypopigmentation macules.

Defects in KRT5 are the cause of Dowling-Degos disease (DDD) [MIM:179850]; also known as Dowling-Degos-Kitamura disease or reticulate acropigmentation of Kitamura. DDD is an autosomal dominant genodermatosis. Affected individuals develop a postpubertal reticulate hyperpigmentation that is progressive and disfiguring, and small hyperkeratotic dark brown papules that affect mainly the flexures and great skin folds. Patients usually show no abnormalities of the hair or nails.

#### Sequence similarities

Belongs to the intermediate filament family.

# Images

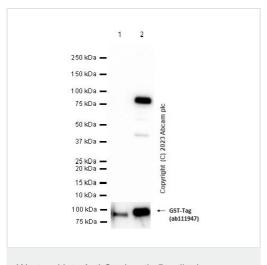


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586) This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).

IHC image of Cytokeratin 5 staining in a section of formalin-fixed paraffin-embedded normal human skin\* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with <a href="mailto:ab52635">ab52635</a>, 0.1 ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586) **All lanes :** Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635) at 1/1 dilution

**Lane 1 :** N-GST tagged full-length recombinant human Cytokeratin 6A protein, 10 ng

**Lane 2 :** N-GST tagged full-length recombinant human Cytokeratin 5 protein, 10 ng

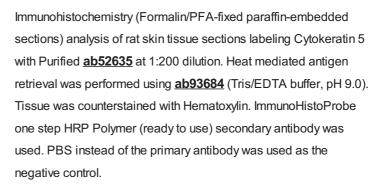
### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 62 kDa **Observed band size:** 87 kDa

Exposure time: 10 seconds

Blocking buffer: 5% NFDM /TBST.

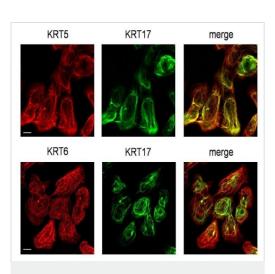


This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody

[EP1601Y] - BSA and Azide free (ab214586)



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

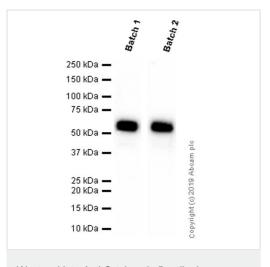
Image from Khanom R. et al PLoS One. 2016 Aug 11;11(8):e0161163. doi: 10.1371/journal.pone.0161163. eCollection 2016.

# Colocalization of KRT5, KRT6 and KRT17 in HSC3 cells

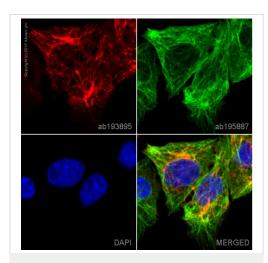
Immunocytochemistry in HSC3 (human oral squamous carcinoma cell line) cells. Scale bar, 10  $\mu m$ .

(Taken from Figure S3 of Khanom et al)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52635</u>).



Western blot - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586) This data was developed using <u>ab52635</u>, the same antibody clone in a different buffer formulation. Different batches of <u>ab52635</u> were tested on Rat skin lysate at 1.0  $\mu$ g/ml. 15  $\mu$ g of lysate was loaded in each lane. Bands observed at 62 kDa.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

ab193894 ab195889

Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

Clone EP1601Y (ab214586) has been successfully conjugated by Abcam. This image was generated using Anti-Cytokeratin 5 antibody [EP1601Y] (Alexa Fluor® 647). Please refer to **ab193895** for protocol details.

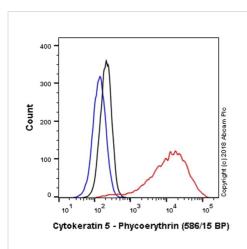
<u>ab193895</u> staining Cytokeratin 5 in A431 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab193895</u> at 1/100 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in A431 cells fixed with 100% methanol (5min).

Clone EP1601Y (ab214586) has been successfully conjugated by Abcam. This image was generated using Anti-Cytokeratin 5 antibody [EP1601Y] (Alexa Fluor® 488). Please refer to <a href="mailto:ab193894">ab193894</a> for protocol details.

**ab193894** staining Cytokeratin 5 in HACAT cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 10% normal goat serum in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab193894** at 1/200 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 594), at 1/200 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

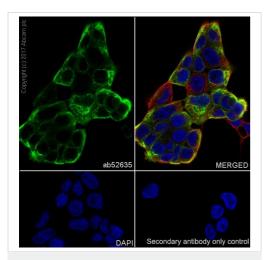
Clone EP1601Y (ab214586) has been successfully conjugated by Abcam. This image was generated using Anti-Cytokeratin 5 antibody [EP1601Y] (PE). Please refer to <a href="mailto:ab224985"><u>ab224985</u></a> for protocol details.

Overlay histogram showing A431 cells stained with <u>ab224985</u> (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (<u>ab224985</u>, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (<u>ab209478</u>) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

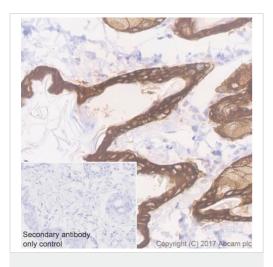
This antibody gave a positive signal in A431 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling Cytokeratin 6 with Purified <a href="mailto:ab52635">ab52635</a> at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor ® 594) 1:200 (2.5 µg/ml). <a href="mailto:ab150077">ab150077</a> Goat anti rabbit lgG(Alexa Fluor ® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

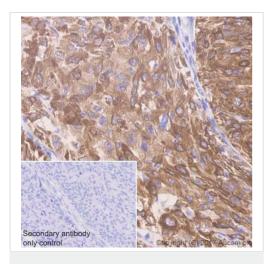
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse skin tissue sections labeling Cytokeratin 5 with Purified <a href="mailto:ab52635">ab52635</a> at 1:200 dilution. Heat mediated antigen retrieval was performed using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52635</u>).

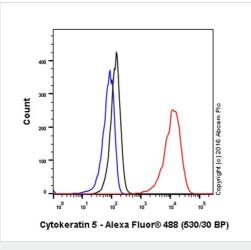


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody

[EP1601Y] - BSA and Azide free (ab214586)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue sections labeling Cytokeratin 5 with Purified <a href="mailto:ab52635">ab52635</a> at 1:200 dilution. Heat mediated antigen retrieval was performed using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Flow Cytometry (Intracellular) - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labelling Cytokeratin 5 with purified <u>ab52635</u> at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor<sup>®</sup>488-conjugated goat anti-rabbit lgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

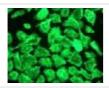
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52635</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

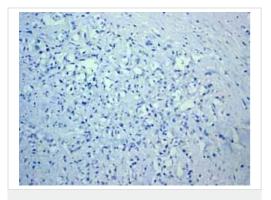
Human transitional urinary bladder carcinoma stained with unpurified **ab52635** at 1/100 - 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586) A431 cells stained with unpurified ab52635 at 1/100 - 1/250

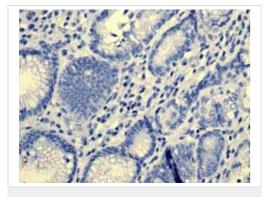
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52635</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

Unpurified <u>ab52635</u> showing negative staining in ductal breast carcinoma tissue.

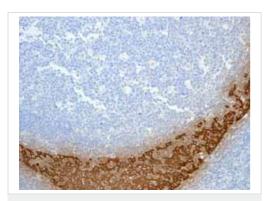
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

Unpurified <u>ab52635</u> showing negative staining in stomach adenocarcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

Unpurified  $\underline{ab52635}$  showing positive staining in normal tonsil squamous cells tissue.

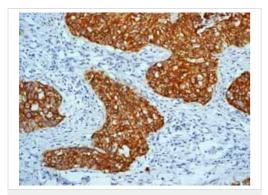
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52635).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody
[EP1601Y] - BSA and Azide free (ab214586)

Unpurified <u>ab52635</u> showing positive staining in squamous cell lung carcinoma tissue.

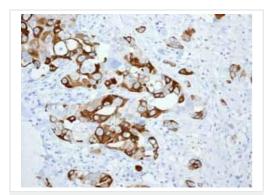
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52635</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

This IHC data was generated using the same anti-Cytokeratin 5 antibody clone, EP1601Y, in a different buffer formulation (cat# ab52635).

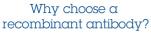
ab52635 showing positive staining in squamous cell cervical carcinoma tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

This IHC data was generated using the same anti-Cytokeratin 5 antibody clone, EP1601Y, in a different buffer formulation (cat# ab52635).

ab52635 showing positive staining in basal cell breast carcinoma tissue.





Long-term and scalable supply Recombinant

technology



compliant Animal-free

production

Success from the Ethical standards first experiment Confirmed specificity

Anti-Cytokeratin 5 antibody [EP1601Y] - BSA and Azide free (ab214586)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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