# abcam

### Product datasheet

## Anti-Cytokeratin 14 antibody [EP1612Y] ab51054



★★★★★ 7 Abreviews 16 References

画像数 9

#### 製品の概要

製品名 Anti-Cytokeratin 14 antibody [EP1612Y]

製品の詳細 Rabbit monoclonal [EP1612Y] to Cytokeratin 14

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF

種交差性 交差種: Human

免疫原 Synthetic peptide within Human Cytokeratin 14 aa 400 to the C-terminus (C terminal). The exact

> sequence is proprietary. Database link: P02533

ポジティブ・コントロール WB: A431 cell lysate. IHC-P: Human skin and human squamous lung carcinoma tissue. ICC/IF:

A431 cells. Flow Cyt (intra): A431 cells. IP: A431 cell lysate

特記事項 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.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

バッファー pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

精製度 Protein A purified

1

**ポリ**Æノ モノクローナル **ウローン名** EP1612Y

ΙgG

アプリケーション

アイソタイプ

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab51054の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	<b>★★★★</b> (2)	1/20000. Detects a band of approximately 48 kDa (predicted molecular weight: 52 kDa).
IP		1/20.
IHC-P	<b>★★★★☆</b> ( <u>2</u> )	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	<b>★★★★☆(1)</b>	1/100.

#### ターゲット情報

機能

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.

組織特異性

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.

関連疾患

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.

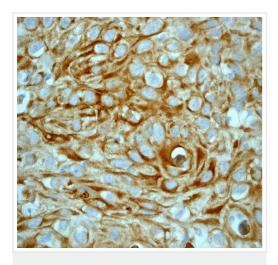
配列類似性

細胞内局在

Belongs to the intermediate filament family.

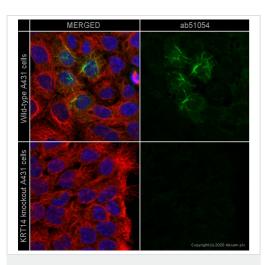
Cytoplasm. Nucleus. Expressed in both as a filamentous pattern.

#### 画像

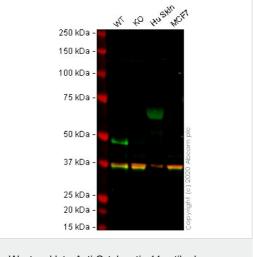


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054)

Immunohistochemical analysis of paraffin-embedded human squamous lung carcinoma tissue sections labeling Cytokeratin 14 with purified ab51054 at 1/100 dilution. ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated antigen retrieval using citrate buffer, pH 6.0).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054) ab51054 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 ab51054 at 1/100 dilution 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 [EP1612Y] (ab51054)

**All lanes :** Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054) at 1/10000 dilution

Lane 1: Wild-type A431 cell lysate

Lane 2: KRT14 knockout A431 cell lysate

Lane 3: Human skin cell lysate

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole

cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

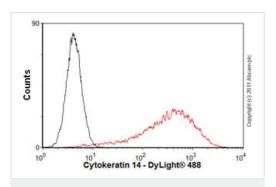
**Predicted band size:** 52 kDa **Observed band size:** 49 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab51054 observed at 49 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab51054 was shown to react with Cytokeratin 14 in wild-type A431 cells in western blot. Loss of signal was observed when KRT14 knockout sample was used. Wild-type A431 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 ab51054 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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.



Immunoprecipitation - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054)



Flow Cytometry (Intracellular) - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054)

Purified ab51054 at 1/20 dilution (0.5 $\mu$ g) immunoprecipitating Cytokeratin 14 in A431 whole cell lysate.

Lane 1 (input): A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate  $10\mu g$ 

Lane 2 (+): ab51054 + A431 whole cell lysate.

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab51054 in A431 whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

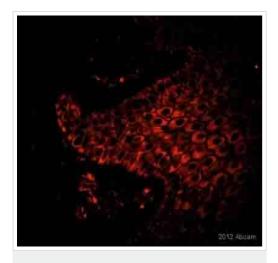
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 48 kDa

Overlay histogram showing A431 (Human epidermoid carcinoma cell line) cells stained with ab51054 (red line).

The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton 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 (ab51054, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight<sup>®</sup> 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was was rabbit lgG (monoclonal)  $(1\mu g/1x10^6 \text{ cells})$  used under the same conditions. Acquisition of >5,000 events was performed.

This antibody gave a positive signal in A431 cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Triton used under the same conditions.

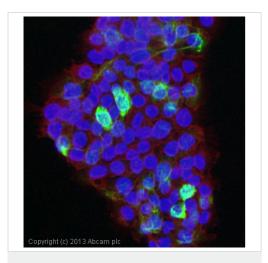


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054)

Image courtesy of an anonymous Abreview.

ab51054 staining Cytokeratin 14 in human skin tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

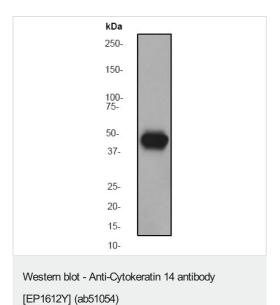
Tissue was fixed in paraformaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer, pH 6.0. Samples were then permeabilized using 0.1% saponin/PBS, blocked with 4% BSA for 30 minutes at 25°C and then incubated with ab51054 at a 1/200 dilution for 16 hours at 4°C. The secondary used was a Texas Red conjugated goat anti-rabbit polyclonal used at a 1/100 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054)

ICC/IF image of <u>ab51504</u> stained A431 (Human epidermoid carcinoma cell line) cells.

The cells were fixed in 100% methanol (5 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab51504, 1/100 dilution) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight<sup>®</sup> 488 goat antirabbit lgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor<sup>®</sup> 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 1.43  $\mu$ M.

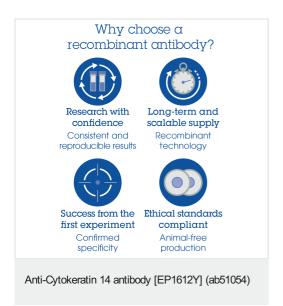


Anti-Cytokeratin 14 antibody [EP1612Y] (ab51054) at 1/20000 dilution + A431 (Human epidermoid carcinoma cell line) cell lysate at 10  $\mu g$ 

#### Secondary

Goat anti-Rabbit-HRP at 1/2000 dilution

**Predicted band size:** 52 kDa **Observed band size:** 48 kDa



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