abcam

Product datasheet

Anti-Involucrin antibody [SY5] ab68

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Overview

Product name	Anti-Involucrin antibody [SY5]
Description	Mouse monoclonal [SY5] to Involucrin
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Immunogen	Tissue, cells or virus corresponding to Human Involucrin. Purified involucrin from human keratinocytes.
Epitope	The epitope maps between codon 421-568 of human involucrin.
Positive control	IHC-P: Human tonsil, skin tissue. Localized to upper spinous and granular layers in normal skin. Flow cyt: A431 cells. ICC/IF: MCF7 cells.
General notes	Involucrin is the differentiation marker of human keratinocytes. **ab81216** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. Staining of formalin-fixed tissues is enhanced by digestion with trypsin or Protease XXV at 1mg/ml PBS for 5 min at 37°C (Note than enzyme digestion is better than citrate buffer based epitope unmasking) 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	Preservative: 0.02% Sodium azide Constituent: 99.98% PBS

Purity	Immunogen affinity purified
Clonality	Monoclonal
Clone number	SY5
Myeloma	P3x63
Isotype	IgG1
Light chain type	unknown

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab68 in the following tested applications.

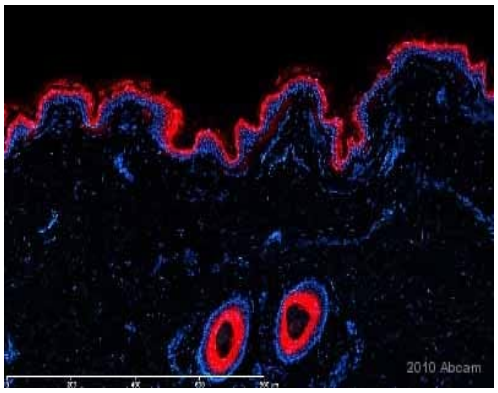
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use a concentration of 1 µg/ml.
IHC-P	★★★☆☆ **(2)**	Use a concentration of 0.1 - 0.2 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function	Part of the insoluble cornified cell envelope (CE) of stratified squamous epithelia.
Tissue specificity	Keratinocytes of epidermis and other stratified squamous epithelia.
Sequence similarities	Belongs to the involucrin family.
Post-translational modifications	Substrate of transglutaminase. Some glutamines and lysines are cross-linked to other involucrin molecules, to other proteins such as keratin, desmoplakin, periplakin and envoplakin, and to lipids like omega-hydroxyceramide.
Cellular localization	Cytoplasm. Constituent of the scaffolding of the cornified envelope.

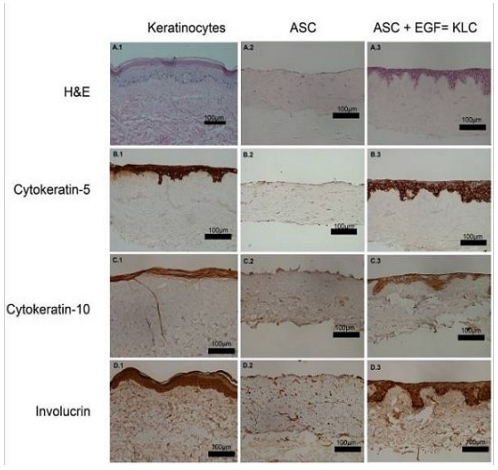
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Involucrin antibody [SY5] (ab68)

This image is courtesy of an anonymous abreview.

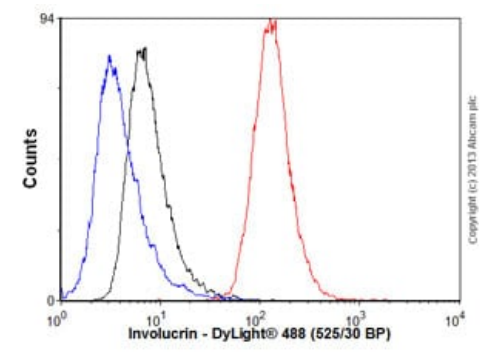
ab68 at a 1/1000 dilution staining Involucrin in human normal skin tissue by Immunohistochemistry (formalin fixed, paraffin embedded sections), incubated for 2 hours at 21°C. Heat mediated antigen retrieval step performed using citrate buffer pH 6.0. Blocked with 10% serum for 30 minutes at 21°C. Secondary used at 1/100 polyclonal Donkey anti-mouse IgG conjugated to Alexa Fluor 594 (red).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Involucrin antibody [SY5] (ab68)

KLC (keratinocyte-like cells) have the capacity to stratify

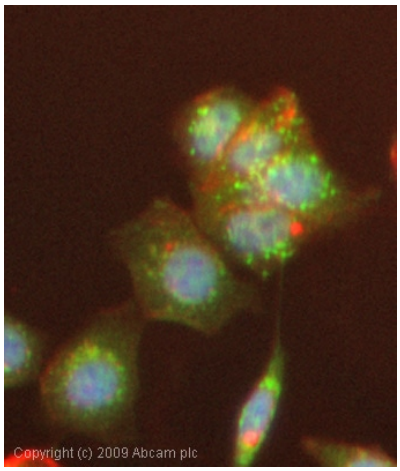
Human skin was decellularized and confirmed complete cell removal by H&E staining. ASC (human adipose-derived stem cells) were seeded on the epithelial side of human dermal decellularized matrices in the presence or absence of EGF (0.005 µg/mL). Human dermal decellularized matrices seeded with keratinocytes were used as controls. All three groups were kept in the same media conditions and lifted to an air-liquid interphase at the same time. Histological analysis revealed the presence of several layers of KLC with an organization similar to that of keratinocytes (A.1and 3). Surprisingly, ASC without EGF not only did not transdifferentiate into KLC, but instead migrated inside the matrix (A.2). Immunohistochemical analysis revealed a positive staining of cytokeratin-5 (B.3), cytokeratin-10 (C.3) and I**nvolucrin** (D.3) in stratified KLC matrices comparable to that of the stratified keratinocyte matrix (A, B, C and D.1). No positive staining was found in the ASC seeded matrices (A, B, C and D.2).



Flow Cytometry - Anti-Involucrin antibody [SY5] (ab68)

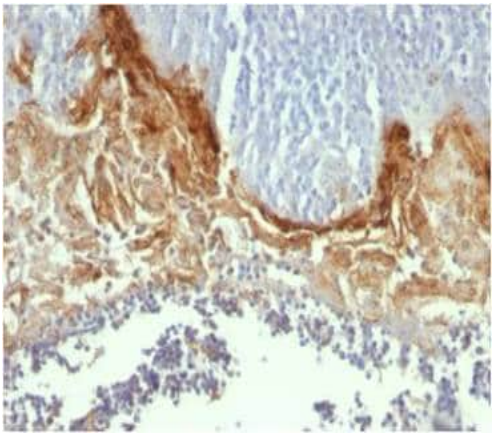
Overlay histogram showing A431 cells stained with ab68 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab68, 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. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000

events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in A431 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Involucrin antibody [SY5] (ab68)

ICC/IF image of ab68 stained MCF7 cells. The cells were 4% formaldehyde fixed (10 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 (ab68, 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Involucrin antibody [SY5] (ab68)

Immunohistochemical analysis of Formalin fixed paraffin embedded human tonsil section labelling Involucrin with ab68 at dilution of 0.1 ug/mL.

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