Product datasheet

Anti-Involucrin antibody [SY5] ab68

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: Pure 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.
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: 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.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐ (2)</td>
<td>Use a concentration of 0.1 - 0.2 µg/ml.</td>
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<tr>
<td>Flow Cyt</td>
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<td>Use 1µg for 10⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
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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
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).

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.1 and 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 Involucrin (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).

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/1x10⁶ 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/1x10⁶ 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.

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.

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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