abcam

Product datasheet

# Anti-AICDA antibody [EPR23436-45] - ChIP Grade ab269454

敲除验证 重组 RabMAb

1 Abreviews 12 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-AICDA抗体[EPR23436-45] - ChIP Grade |
| **描述** | 兔单克隆抗体[EPR23436-45] to AICDA - ChIP Grade |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** Flow Cyt (Intra), IHC-P, ICC/IF, IP, WB, ChIP |
| **种属反应性** | **与反应:** Human |
| **免疫原** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | WB: Ramos,NAMALWA and Raji lysates. IHC-P: Human tonsil, Human Hodgkin lymphoma and Human diffuse large B-cell lymphoma tissues. ICC/IF: NAMALWA cells. Flow Cyt (intra): NAMALWA cells. IP: Ramos and NAMALWA cells.ChIP: Chromatin prepared from Ramos cells. |
| **常规说明** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 性能

| | |
|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **存储溶液** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | EPR23436-45 |

**同种型** IgG

## 应用

**The Abpromise guarantee** Abpromise™承诺保证使用ab269454于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **Flow Cyt (Intra)** | | 1/500. |
| **IHC-P** | ★★★★★ (1) | 1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ICC/IF** | | 1/50. |
| **IP** | | 1/30. |
| **WB** | | 1/1000. Detects a band of approximately 24 kDa (predicted molecular weight: 23 kDa). |
| **ChIP** | | Use 5 µg for 25 µg of chromatin. |

## 靶标

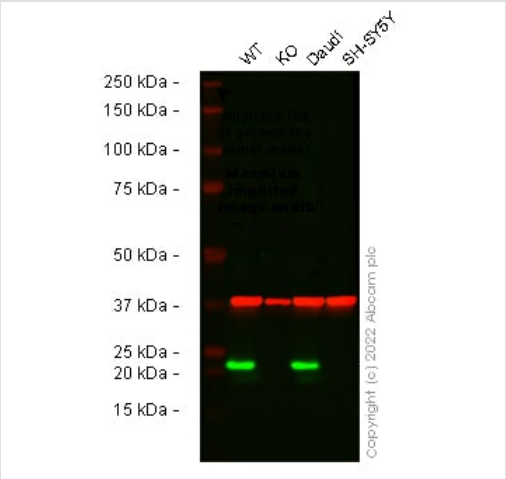
**功能** RNA-editing deaminase involved in somatic hypermutation, gene conversion, and class-switch recombination. Required for several crucial steps of B-cell terminal differentiation necessary for efficient antibody responses.

**组织特异性** Strongly expressed in lymph nodes and tonsils.

**疾病相关** Defects in AICDA are the cause of hyper-IgM immunodeficiency syndrome type 2 (HIGM2) [MIM:605258]; also known as hyper-IgM syndrome 2. HIGM2 is an autosomal recessive disorder characterized by normal or elevated serum IgM levels with absence of IgG, IgA, and IgE, resulting in a profound susceptibility to bacterial infections. HIGM2 causes the absence of Ig class switch recombination (CSR), the lack of Ig somatic hypermutations, and lymph node hyperplasia caused by the presence of giant germinal centers.

**序列相似性** Belongs to the cytidine and deoxycytidylate deaminase family.

## 图片




Western blot - Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454)

**All lanes :** Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454) at 1/1000 dilution

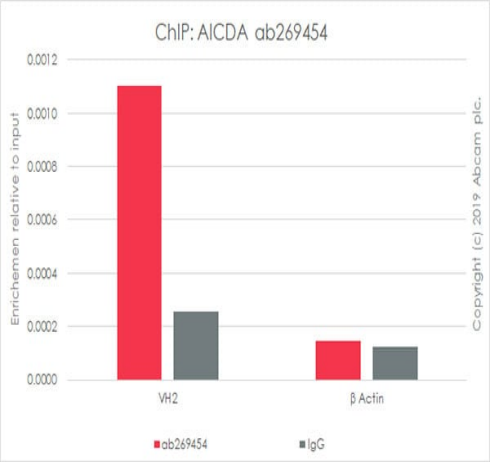
**Lane 1 :** Wild-type Raji cell lysate
**Lane 2 :** AICDA knockout Raji cell lysate
**Lane 3 :** Daudi cell lysate
**Lane 4 :** SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 23 kDa
**Observed band size:** 22 kDa

False colour image of Western blot: Anti-AICDA antibody [EPR23436-45] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab269454 was shown to bind specifically to AICDA. A band was observed at 22 kDa in wild-type Raji cell lysates with no signal observed at this size in AICDA knockout cell line **ab277185** (knockout cell lysate **ab277227**). To generate this image, wild-type and AICDA knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



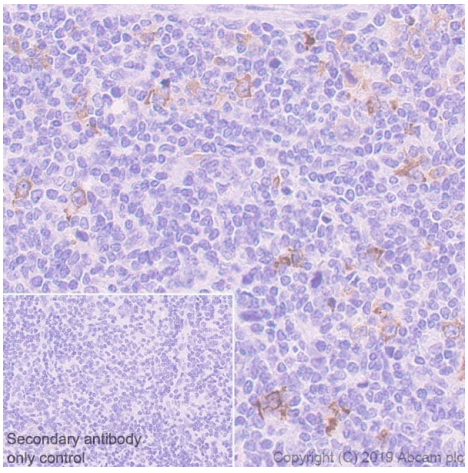

ChIP - Anti-AICDA antibody [EPR23436-45] (ab269454)

Chromatin was prepared from Ramos cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab269454 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

Primers and probes are commercial primers from paper: PMC2905439

***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**



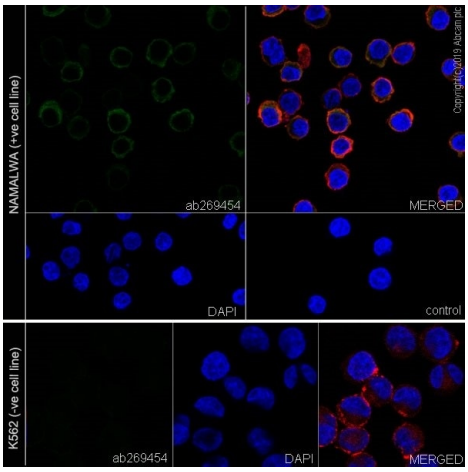

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AICDA antibody [EPR23436-45] (ab269454)

Immunohistochemical analysis of paraffin-embedded Human diffuse large B-cell lymphoma tissue labeling AICDA with ab269454 at 1/4000 dilution (0.12ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Mainly cytoplasmic staining (weak nuclear staining) in part of tumor cells of human diffuse large B-cell lymphoma (PMID: 29251015).

The section was incubated with **ab255611** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

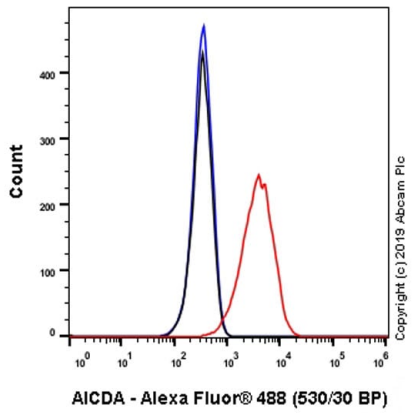
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.




Immunocytochemistry/ Immunofluorescence - Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454)

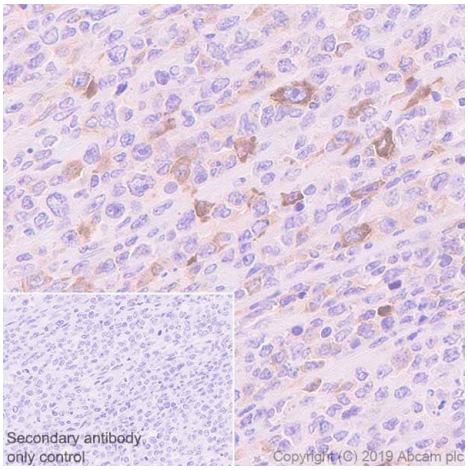
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NAMALWA (human Burkitt's lymphoma B lymphocyte) and K-562 (human chronic myelogenous leukemia lymphoblast) cells labelling AICDA with ab269454 at 1/50 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in NAMALWA cell line. **Negative control:** K-562 cell line (PMID: 27217538). **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.




Flow Cytometry (Intracellular) - Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized Ramos (human Burkitt's lymphoma B lymphocyte) cells labelling AICDA with ab269454 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



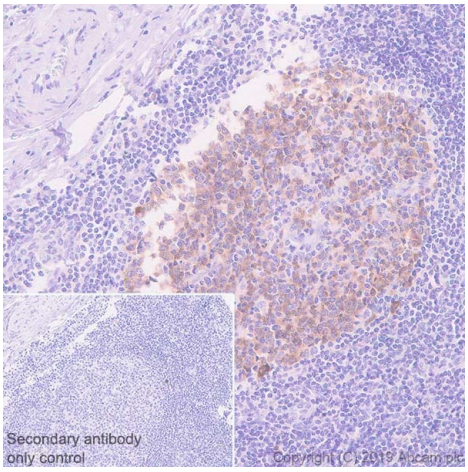

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AICDA antibody [EPR23436-45] (ab269454)

Immunohistochemical analysis of paraffin-embedded Human Hodgkin lymphoma tissue labeling AICDA with ab269454 at 1/4000 dilution (0.12ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Mainly cytoplasmic staining (weak nuclear staining) in part of tumor cells of human Hodgkin lymphoma (PMID: 15732141).

The section was incubated with **ab255611** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



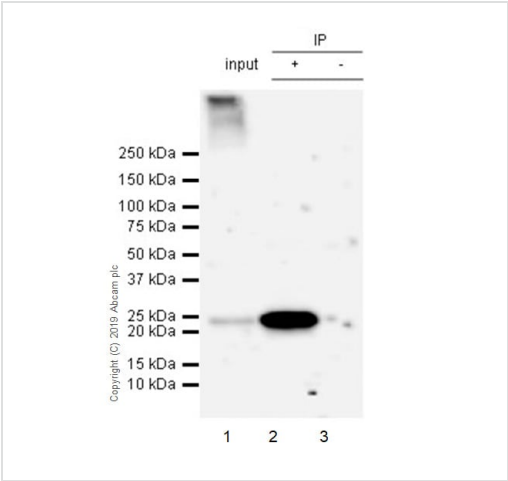

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AICDA antibody [EPR23436-45] (ab269454)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling AICDA with ab269454 at 1/4000 dilution (0.12ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Mainly cytoplasmic staining (weak nuclear staining) in germinal center cells of human tonsil (PMID:23877718, 15732141, PMID: 29251015).

The section was incubated with **ab255611** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.




Immunoprecipitation - Anti-AICDA antibody [EPR23436-45] (ab269454)

AICDA was immunoprecipitated from 0.35 mg NAMALWA (human Burkitt's lymphoma B lymphocyte) whole cell lysate with ab269454 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab269454 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

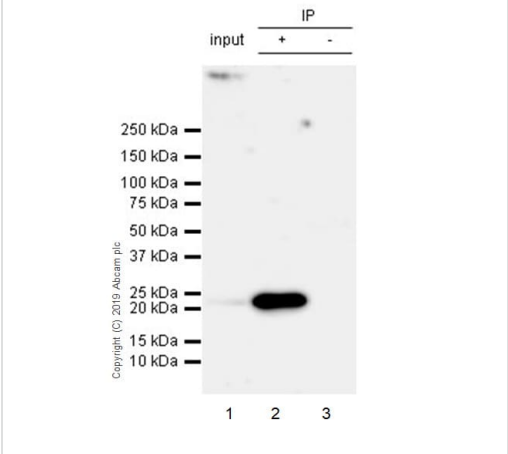
Lane 1: NAMALWA whole cell lysate 10ug.

Lane 2: ab269454 IP in NAMALWA whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab269454 in NAMALWA whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 90 seconds.




Immunoprecipitation - Anti-AICDA antibody [EPR23436-45] (ab269454)

AICDA was immunoprecipitated from 0.35 mg Ramos (human Burkitt's lymphoma B lymphocyte) whole cell lysate with ab269454 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab269454 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

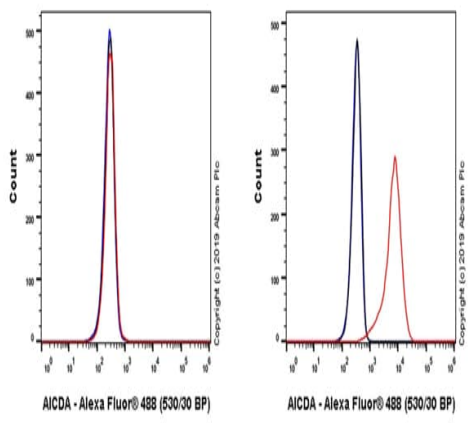
Lane 1: Ramos whole cell lysate 10ug.

Lane 2: ab269454 IP in Ramos whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab269454 in Ramos whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

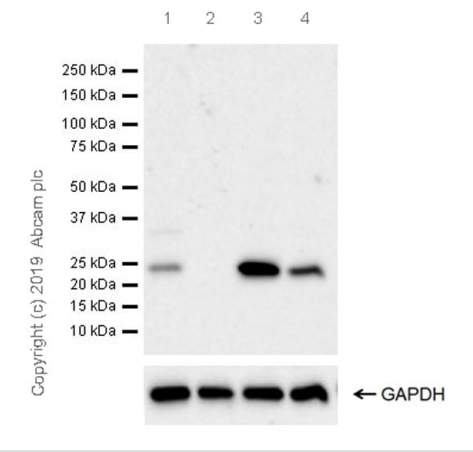
Exposure time: 90 seconds.




Flow Cytometry (Intracellular) - Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized K-562 (human chronic myelogenous leukemia lymphoblast, Left) / NAMALWA (human Burkitt's lymphoma B lymphocyte, Right) cells labelling AICDA with ab269454 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

**Negative control:** K-562 cell line (PMID: 27217538).




Western blot - Anti-AICDA antibody [EPR23436-45] (ab269454)

**All lanes :** Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454) at 1/1000 dilution

**Lane 1 :** Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate
**Lane 2 :** K-562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate
**Lane 3 :** NAMALWA (human Burkitt's lymphoma B lymphocyte), whole cell lysate
**Lane 4 :** Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

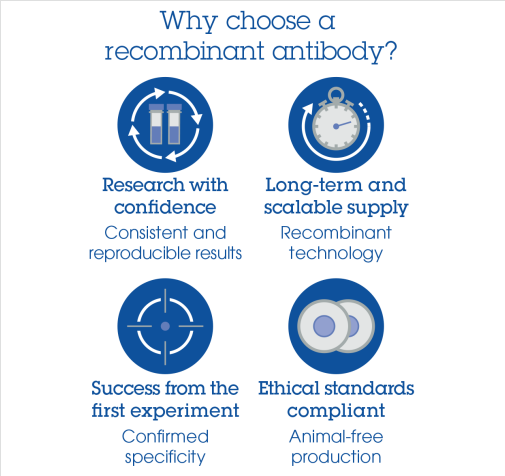
**Predicted band size:** 23 kDa
**Observed band size:** 24 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 114 seconds.

**Negative control**: K-562 (PMID: 27217538).

The expression profile observed is consistent with what has been described in the literature (PMID: 27217538).




Anti-AICDA antibody [EPR23436-45] - ChIP Grade (ab269454)

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

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