# abcam

# Product datasheet

# Anti-CHD4 antibody [EPR22953-38] - ChIP Grade ab240640

Recombinant RabMAb

# 7 Images

#### Overview

**Product name** Anti-CHD4 antibody [EPR22953-38] - ChIP Grade

**Description** Rabbit monoclonal [EPR22953-38] to CHD4 - ChIP Grade

**Host species** Rabbit

**Tested applications** Suitable for: IP, Flow Cyt (Intra), WB, ICC/IF, ChIP

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse, Human

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

Positive control WB: NCCIT, K562, HeLa, HEK-293 and RAW264.7 lysates. ICC/IF: HeLa and NIH/3T3 cells.

Flow Cyt (intra): HeLa cells. IP: NCCIT cells. ChIP: Chromatin prepared from K562 cells.

General notes 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**.

## **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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Protein A purified **Purity** 

Clonality Monoclonal

Clone number EPR22953-38

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab240640 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
IP		1/30.
Flow Cyt (Intra)		1/600.
WB		1/1000. Predicted molecular weight: 218 kDa.
ICC/IF		1/500.
ChIP		Use 5 µg for 25 µg of chromatin.

**Application notes** Is unsuitable for IHC-P.

**Target** 

Function Component of the histone deacetylase NuRD complex which participates in the remodeling of

chromatin by deacetylating histones.

**Sequence similarities**Belongs to the SNF2/RAD54 helicase family.

Contains 2 chromo domains.

Contains 1 helicase ATP-binding domain. Contains 1 helicase C-terminal domain. Contains 2 PHD-type zinc fingers.

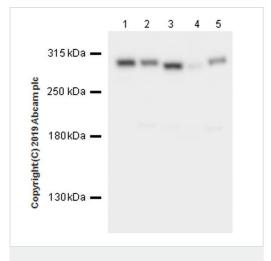
Post-translational modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

**Cellular localization** 

Nucleus. Cytoplasm > cytoskeleton > centrosome. Associates with centrosomes in interphase.

#### **Images**



Western blot - Anti-CHD4 antibody [EPR22953-38] (ab240640)

**All lanes :** Anti-CHD4 antibody [EPR22953-38] - ChIP Grade (ab240640) at 1/1000 dilution

**Lane 1 :** NCCIT (human pluripotent embryonic carcinoma epithelial cell), whole cell lysate

**Lane 2 :** K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate

Lane 3: HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 4: HEK-293 (human embryonic kidney epithelial cell), whole cell lysate

**Lane 5 :** RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage), whole cell lysate

Lysates/proteins at 20 µg per lane.

## Secondary

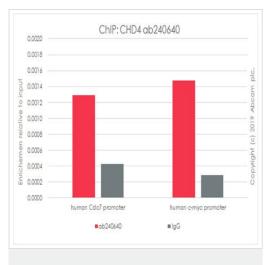
**All lanes :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at 1/100000 dilution

**Predicted band size:** 218 kDa **Observed band size:** 280 kDa

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: PMID: 28572115).

Exposure time: 1 second.

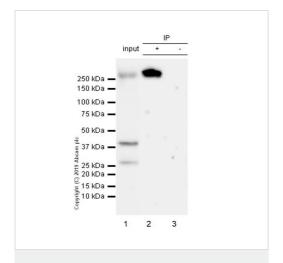


ChIP - Anti-CHD4 antibody [EPR22953-38] (ab240640)

Chromatin was prepared from K-562 cells according to the Abcam Dual-X-ChIP protocol\*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25  $\mu$ g of chromatin, 5  $\mu$ g of ab240640 (red), or 5  $\mu$ g of rabbit normal IgG <u>ab172730</u> (gray) and 20  $\mu$ I 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 from paper PMID: 23505388.

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



Immunoprecipitation - Anti-CHD4 antibody [EPR22953-38] (ab240640)

CHD4 was immunoprecipitated from 0.35 mg NCCIT (Human pluripotent embryonic carcinoma epithelial cell) whole cell lysate 10ug with ab240640 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab240640 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

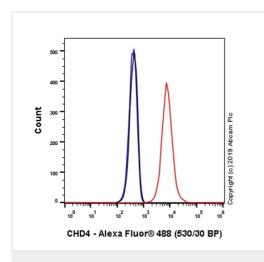
Lane 1: NCCIT whole cell lysate 10ug.

Lane 2: ab240640 IP in NCCIT whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab240640 in NCCIT whole cell lysate.

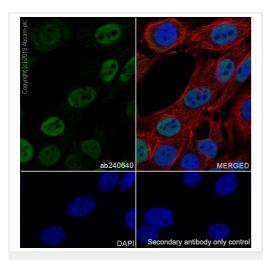
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 min.



Flow Cytometry (Intracellular) - Anti-CHD4 antibody [EPR22953-38] - ChIP Grade (ab240640)

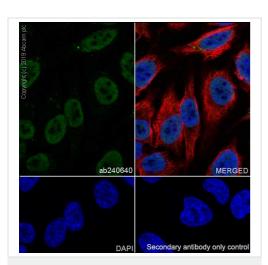
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling CHD4 with ab240640 at 1/600 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-CHD4 antibody [EPR22953-38] (ab240640)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling CHD4 with ab240640 at 1/500 dilution, followed by **ab150077** AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing strong nuclear staining and weak cytoplasmic staining in NIH/3T3 cell line is observed. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

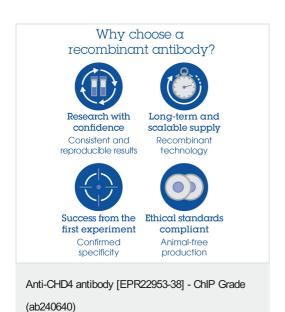
Secondary antibody only control: Secondary antibody is <u>ab150077</u>
AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-CHD4 antibody [EPR22953-38] (ab240640)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling CHD4 with ab240640 at 1/500 dilution, followed by **ab150077** AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing strong nuclear staining and weak cytoplasmic staining in HeLa cell line is observed. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

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



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