abcam

Product datasheet

Anti-EED antibody [EPR23043-5] - ChIP Grade ab240650

KO VALIDATED Recombinant RabMAb

3 Abreviews 6 Images

Overview

Product name	Anti-EED antibody [EPR23043-5] - ChIP Grade
Description	Rabbit monoclonal [EPR23043-5] to EED - ChIP Grade
Host species	Rabbit
Specificity	ab240650 detects an unknown band close to the target bands in cytoplasm.
Tested applications	**Suitable for:** ChIP, ChIP-sequencing, WB, IP **Unsuitable for:** ICC/IF or IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild type HAP1, K562, 293T, NIH/3T3 and C2C12 lysates. IP: K562 cells. ChIP: Chromatin prepared from NT2/D1 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	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23043-5

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab240650 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
ChIP	★★★★★ (1)	Use 5 µg for 25 µg of chromatin.
ChIP-sequencing		Use at an assay dependent concentration. Use at 0.1 uL/ug chromatin.
WB	★★★★★ (2)	1/1000. Predicted molecular weight: 50 kDa.
IP		1/30.

Application notes Is unsuitable for ICC/IF or IHC-P.

Target

Function Polycomb group (PcG) protein. Component of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' and 'Lys-27' of histone H3, leading to transcriptional repression of the affected target gene. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1 and CDKN2A.

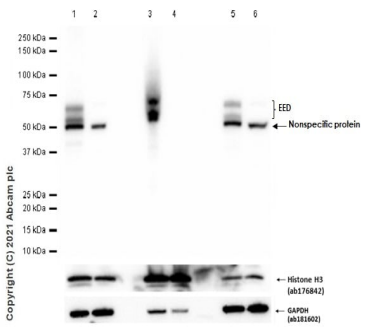
Tissue specificity Expressed in brain, colon, heart, kidney, liver, lung, muscle, ovary, peripheral blood leukocytes, pancreas, placenta, prostate, spleen, small intestine, testis, thymus and uterus. Appears to be overexpressed in breast and colon cancer.

Sequence similarities Belongs to the WD repeat ESC family.
Contains 7 WD repeats.

Developmental stage Expression peaks at the G1/S phase boundary.

Cellular localization Nucleus. Chromosome. Transiently colocalizes with XIST at inactive X chromosomes.

Images



Western blot - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

All lanes : Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650) at 1/1000 dilution

Lane 1 : Wild type HAP1 whole cell lysate
Lane 2 : EED knockout HAP1 whole cell lysate
Lane 3 : Wild type HAP1 nuclear fraction lysate
Lane 4 : EED knockout HAP1 nuclear fraction lysate
Lane 5 : Wild type HAP1 cytoplasmic lysate
Lane 6 : EED knockout HAP1 cytoplasmic lysate

Lysates/proteins at 20 µg per lane.

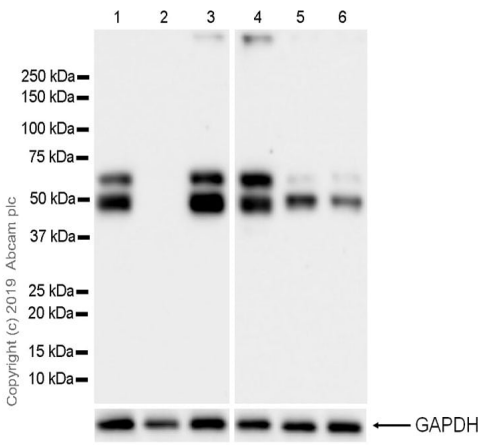
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 50 kDa
Observed band size: 50-70 kDa

Exposure time: 3 seconds

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

ab240650 detects an unknown band close to the target bands in cytoplasm.



Western blot - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

All lanes : Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650) at 1/1000 dilution

Lane 1 : Wild type HAP1 whole cell lysate
Lane 2 : EED knockout HAP1 whole cell lysate
Lane 3 : K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate
Lane 4 : 293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 5 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 6 : C2C12 (mouse myoblasts myoblast), whole cell lysate

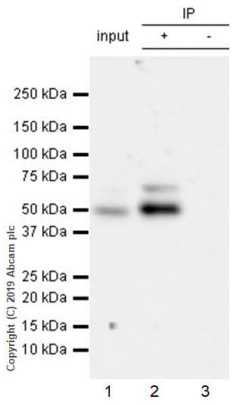
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 50 kDa
Observed band size: 50-70 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST ab240650 was shown to specifically react with EED in wild-type HAP1 cells as signal was lost in EED knockout cells. Wild-type and EED knockout samples were subjected to SDS-PAGE. ab240650 and ab181602 (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID:27578866, 9584199). EED cDNA encodes 441-aa-long protein and 535-aa-long protein.

Exposure time: Lanes 1-3: 15 seconds Lanes 4-6: 37 seconds



Immunoprecipitation - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

EED was immunoprecipitated from 0.35 mg K562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate with ab240650 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab240650 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

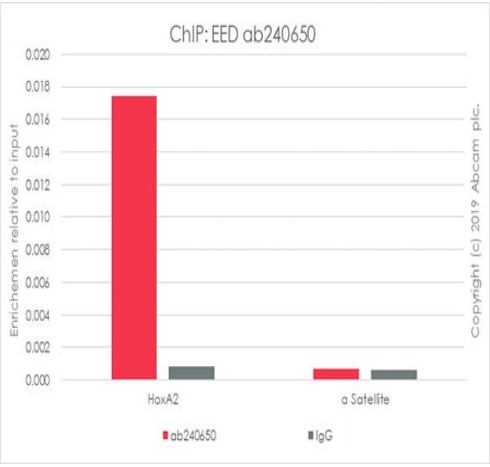
Lane 1: K562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate 10 µg

Lane 2: ab240650 IP in K562 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab240650 in K562 whole cell lysate

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

Exposure time: 1 min



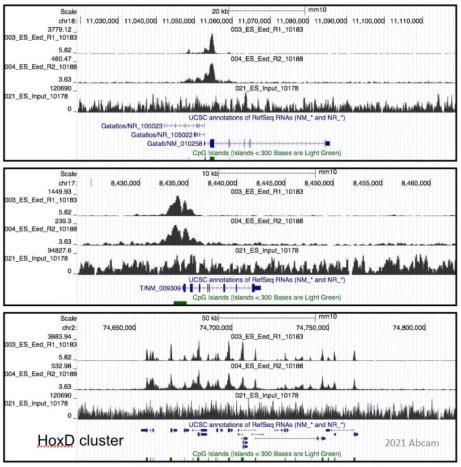
ChIP - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

Chromatin was prepared from NT2/D1 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 µg of chromatin, 5 µg of ab240650 (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 Millipore (Cat. No.: 17-10034) and CST (85322S)

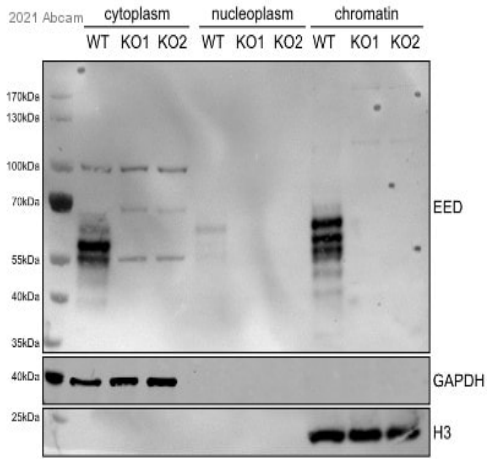
*https://www.abcam.com/resources?keywords=X%20ChIP%20protocol



ChIP-sequencing - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

This image is courtesy of an Abreview submitted by Ivano Mocavini.

ChIP sequencing analysis of chromatin from Mouse Embryonic Stem Cells with ab240650 at 0.1 µL/µg chromatin. Cross linking was performed for 10 minutes with 1% PFA. Primary incubation was for 16 hours at 4°C in a dilution buffer containing 20mM Tris at pH8, 1.1mM EDTA, 1.1% triton, and 167mM NaCl.



Western blot - Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650)

This image is courtesy of an Abreview submitted by Ivano Mocavini.

All lanes : Anti-EED antibody [EPR23043-5] - ChIP Grade (ab240650) at 1/1000 dilution

Lane 1 : Wild type Mouse Embryonic Stem Cell (mESC) cytoplasmic extract
Lane 2 : EED knockout (KO1) mESC cytoplasmic extract
Lane 3 : EED knockout (KO2) mESC cytoplasmic extract
Lane 4 : Wild type mESC nucleoplasm extract
Lane 5 : EED knockout (KO1) mESC nucleoplasm extract
Lane 6 : EED knockout (KO2) mESC nucleoplasm extract
Lane 7 : Wild type mESC chromatin extract
Lane 8 : EED knockout (KO1) mESC chromatin extract
Lane 9 : EED knockout (KO2) mESC chromatin extract

Secondary
All lanes : Goat anti-rabbit antibody conjugated to HRP at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 40,47,55,60,65 kDa

Exposure time: 30 seconds

Additional bands at: 100 kDa, 70 kDa and 55 kDa in the cytoplasmic fraction (all possible non-specific binding)

This blot was produced using a 4-12% Bis-tris gel under reducing denaturing conditions. Following transfer, the membrane was blocked for 30 minutes at room temperature using 5% Milk before being incubated with ab240650 for 16 hours at 4°C.

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