abcam

Product datasheet

Anti-MBD3 antibody [EPR9913] - ChIP Grade ab157464

KO VALIDATED Recombinant RabMAb

***** 9 Abreviews 18 References 8 Images

Overview

| Product name | Anti-MBD3 antibody [EPR9913] - ChIP Grade |
|---------------------|---|
| Description | Rabbit monoclonal [EPR9913] to MBD3 - ChIP Grade |
| Host species | Rabbit |
| Tested applications | Suitable for: Flow Cyt (Intra), IP, WB, ICC/IF, ChIP Unsuitable for: IHC-P |
| Species reactivity | Reacts with: Mouse, Rat, Cow, Human |
| Immunogen | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| Positive control | HeLa, 293T, fetal brain and Y79 lysates; HeLa cells; Permeabilized 293T 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 $^{	extsf{B}}$ technology is a patented hybridoma-based technology for making rabbit |
| | monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u> . |

| Properties | |
|----------------------|---|
| Form | Liquid |
| Storage instructions | Shipped at 4°C. Store at -20°C. |
| Storage buffer | pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant |
| Purity | Tissue culture supernatant |
| Clonality | Monoclonal |
| Clone number | EPR9913 |
| lsotype | lgG |

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab157464 in the following tested applications.

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

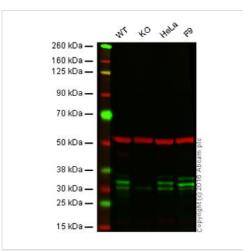
| Application | Abreviews | Notes |
|------------------|---|--|
| Flow Cyt (Intra) | | 1/10 - 1/100. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| IP | ★★★★★ (1) | Use at an assay dependent concentration. |
| WB | \star \star \star \star \star (4) | 1/1000 - 1/5000. Predicted molecular weight: 33 kDa. |
| ICC/IF | ★★★★★ <u>(2)</u> | 1/100 - 1/1000. |
| ChIP | ★★★★★ (1) | Use at an assay dependent concentration. |

Application notes

Is unsuitable for IHC-P.

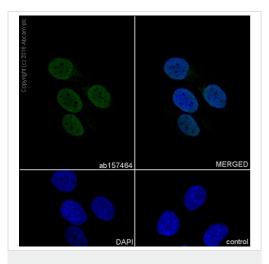
| Target | |
|-----------------------|--|
| Function | Does not bind DNA by itself. Recruits histone deacetylases and DNA methyltransferases. Acts as transcriptional repressor and plays a role in gene silencing. |
| Sequence similarities | Contains 1 MBD (methyl-CpG-binding) domain. |
| Cellular localization | Nucleus. Nuclear, in discrete foci. |

Images



Western blot - Anti-MBD3 antibody [EPR9913] -ChIP Grade (ab157464) Lane 1: Wild-type HAP1 cell lysate (20 µg) Lane 2: ProteinX knockout HAP1 cell lysate (20 µg) Lane 3: Wild-type HAP1 cell lysate (20 µg) Lane 4: ProteinX knockout HAP1 cell lysate (20 µg) Lanes 1 - 4: Merged signal (red and green). Green - ab157464 observed at 33 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab157464 was shown to recognize MBD3 when MBD3 knockout samples were used, along with additional cross-reactive bands. Wild-type and MBD3 knockout samples were subjected to SDS-PAGE. ab157464 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L

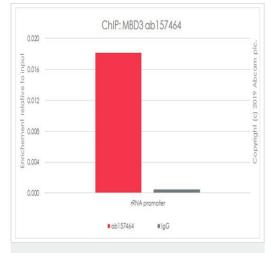


(IRDye[®] 680RD) preadsorbed (<u>**ab216776**</u>) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling MBD3 with purified ab157464 at 1/1000. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor[®] 488conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only

Immunocytochemistry/ Immunofluorescence - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)



ChIP - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

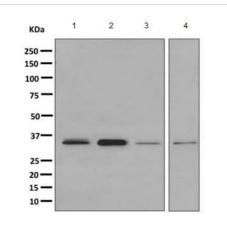
Chromatin was prepared from HeLa cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab157464 (red), and 20 μ l of Protein A/G sepharose beads. 5 μ g of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

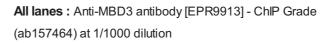
Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources?

keywords=X%20ChIP%20protocol



Western blot - Anti-MBD3 antibody [EPR9913] -ChIP Grade (ab157464)



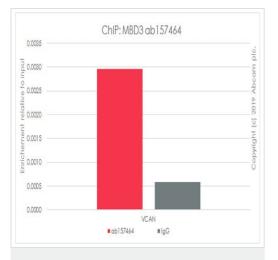
Lane 1 : HeLa cell lysate Lane 2 : 293T cell lysate Lane 3 : Fetal brain lysate Lane 4 : Y79 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 33 kDa



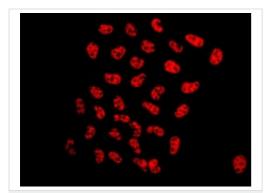
ChIP - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

Chromatin was prepared from NIH/3T3 cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab157464 (red), and 20 μ l of Protein A/G sepharose beads. 5 μ g of rabbit normal lgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

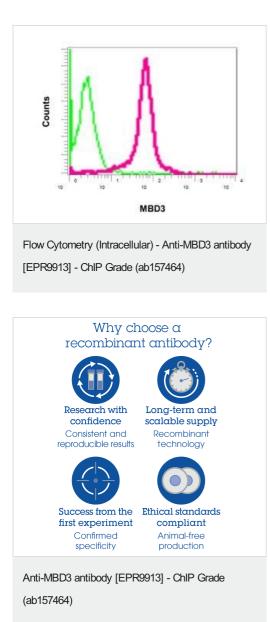
Primers and probes are located in the first kb of the transcribed region.

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Immunocytochemistry/ Immunofluorescence - Anti-MBD3 antibody [EPR9913] - ChIP Grade (ab157464)

Immunofluorescent analysis of HeLa cells labeling MBD3 with ab157464 at 1/100 dilution.



Intracellular flow cytometric analysis of permeabilized 293T cells labeling MBD3 with ab157464 at 1/10 dilution (red) compared to a rabbit IgG negative control (green).

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