Affinity-Purified Goat Anti-human/mouse MBD4 Antibody

ORDERING INFORMATION

Catalog Number: AF5935

Lot Number: CDIE01

Size: 100 μg

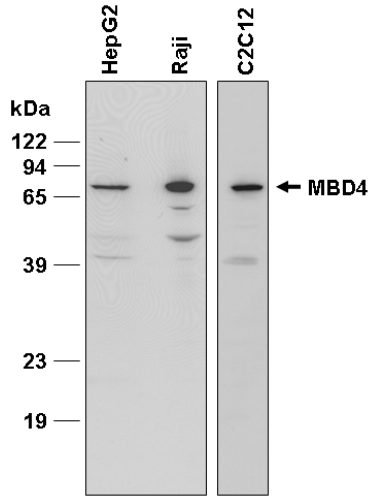
Storage: -20° C

Specificity: human/mouse MBD4

Immunogen: *E. coli*-derived rhMBD4 (aa 382 - 580)

Ig Type: goat IgG

Application: Western blot



Detection of MBD4 with AF5935.
Lysates from human HepG2, human Raji and mouse C2C12 cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1.0 μg/mL goat anti-MBD4, as described in *Protocols for Immunoblotting*.

Background

MBD4 (Methyl CpG-binding domain protein 4; also MED1) is a 68 kDa member of the methyl CpG binding protein family. It is expressed in non-germline cells, and serves at least two functions: one, it interacts with FADD to up- or down-regulate apoptosis; and two, it acts as a DNA N-glycosylase, excising improper chromosomal thymines that are created through the spontaneous deamination of methylcytosine to thymine. Human MDB4 is 580 amino acids (aa) in length and contains one DNA-binding MBD domain (aa 76 - 148) and one endonuclease domain (aa 455 - 580). Over aa 382 - 580, human MBD4 shares 90% aa identity with mouse MBD4. Cells from mice that are MBD4-deficient display altered apoptosis following DNA damage.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human MBD4 (rhMBD4; aa 382 - 580; Accession # O95243). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein.

Formulation

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human and mouse MBD4 at 68 kDa using Western blots.

Application

Western blot - An antibody concentration of 1.0 μg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 μg/mL goat anti-human/mouse MBD4.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL.

Optimal dilutions should be determined by each laboratory for each application.

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