

## ORDERING INFORMATION

**Catalog Number:** AF5935

**Lot Number:** CDIE01

**Size:** 100 µg

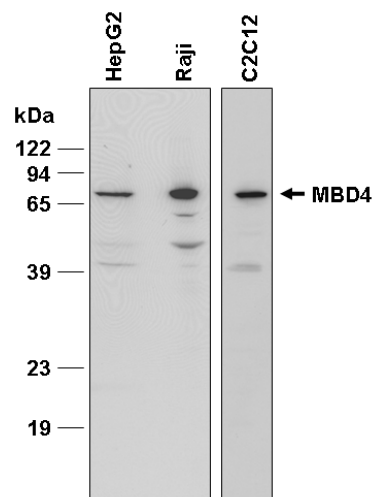
**Storage:** -20° C

**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 MBD4 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<sub>3</sub>.

## 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

25 mM Tris, pH 7.4  
0.15 M NaCl  
0.1% Tween® 20

#### Blocking Solution

5% nonfat dry milk  
in Blotting Buffer  
Adjust pH to 7.4

#### Antibody Solution

5% nonfat dry milk  
in Blotting Buffer  
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 x 10<sup>6</sup> - 1 x 10<sup>7</sup> cells per mL.

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