

PRODUCT NAME
H6-GST-MBD fusion protein

Cat.# mbd-001-100 / gst-001-050

Product description

This H6-GST-MBD fusion protein (cat# mbd-001-100) has been extensively validated. It consists of the methyl binding domain (MBD) of human MeCP2, as a C-terminal fusion with Glutathione-S-transferase (GST) containing an N-terminal His₆-tag. The H6-GST-MBD fusion protein can be used to specifically isolate DNA containing methylated CpGs. See overview and protocol below.

GST protein (cat# gst-001-050) can also be purchased, to be used as negative control in the MBD pull-down experiment (see below).

Description	Reference	Comments	Quantity
H6-GST-MBD fusion protein	mbd-001-100	10 mg /ml; MM 36.657 g /mol ; lot# 001	100 µg (10 µl)
GST-protein	gst-001-050	8 mg /ml ; lot# 001	50 µg (6.25 µl)

Format

Purified over a nickel affinity matrix. Supplied in solution in PBS containing 10% glycerol. It is recommended to use a nickel affinity matrix to isolate hexahis-GST-MBD / DNA complexes. The fusion protein should be used in buffers containing at least 0.2 M NaCl as the protein will precipitate at lower ionic strengths. The preparation was scanned spectrophotometrically between 220 and 350 nm, and the resulting spectrum shows a protein preparation free of contaminating bacterial DNA. Electrophoresis on SDS-PAGE indicates that the protein is essentially homogeneous.

Storage

Store at -80°C. Avoid multiple freeze-thaw cycles.

Precautions

This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Overview

Methylation of CpG dinucleotides is generally associated with epigenetic silencing of transcription and is maintained through cellular division. Multiple CpG sequences are rare in mammalian genomes, but frequently occur at the transcriptional start site of active genes, with most clusters of promoter CpGs being hypomethylated [1].

The binding specificity of the H6-GST-MBD fusion protein to un-, hemi- and fully methylated DNA was evaluated using synthetic DNA that either contained three methylated CpGs [GAM3], three hemimethylated CpG's or no methylated CpGs [GAM]. Hemimethylated DNA does not stably interact with the MBD of MeCP2. A single fully methylated CpG is sufficient for the interaction between the H6-GST-MBD fusion protein and methylated DNA, whereas there is little binding to a hemimethylated target sequence [1].

Reference:

1. Kangaspeska S, Stride B, Métivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G. 2008 Transient cyclical methylation of promoter DNA. *Nature* 452(7183):112-5.

Pull-down protocol

Note : You might need to adjust the protocol for small scale preparations or adapt the volumes and amounts to your experiment.

1. Preparation of Genomic DNA from E. coli

1. From a 10 cm² plate, wash cells twice with PBS and add 1 ml of DNAzol (Invitrogen).
2. Incubate for two minutes and transfer the DNAzol to a 2 ml microtube.
3. Add 0.7 ml of isopropanol, mix well and collect the genomic DNA by centrifugation for 10 minutes at full-speed.
4. Wash the pellet twice with 75% ethanol then resuspend the pellet in 0.5 ml of 8 mM NaOH.
5. Incubate overnight at 65°C with shaking to redissolve the DNA. Estimate DNA concentration spectrophotometrically.

2. Bioruptor DNA Shearing

1. Take 20 µg genomic DNA, adjust volume to 200 µl with freshly prepared buffer 20mM Tris pH 8, 100 mM NaCl, 2 mM DTT (add DTT just before use from a 1 M frozen stock).
2. Sonicate 10 min using the Bioruptor. Settings: High; intervals: 0.2 (Cycles of 12 seconds «ON» and 12 seconds «OFF»). Fill the Bioruptor waterbath with ice water up to the mark before use.
3. Check the fragment size on gel, which should be around 300 bp.

3. Binding

1. Take 5 µg of sheared DNA into an Eppendorf tube and add 2 µg of H6-GST-MBD fusion protein (cat# mdb-001-100). You can also use GST (cat# gst-001-050) alone as a control.
2. Adjust the volume to 200 µl with buffer A (0.25M NaCl, 20 mM Tris, pH 8.5).
3. Rotate 30 min at room temperature.
4. Keep the rest of the sheared DNA as input or for further experiments and store it at -20°C.

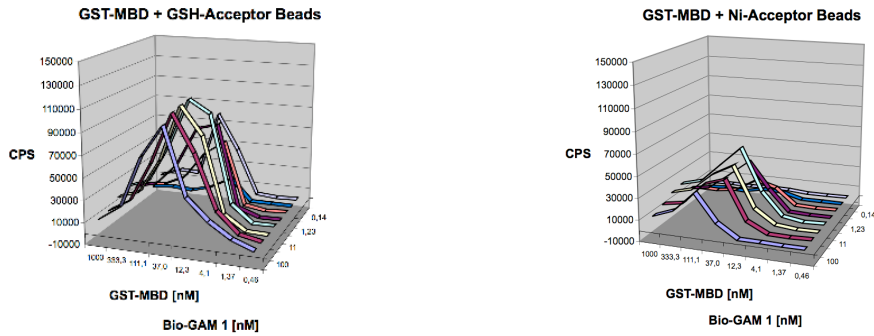
4. Affinity isolation of methylated DNA

1. Add 100 µl of a 50% slurry of NTA-agarose beads (Sigma, cat# P6611) equilibrated in buffer A.
2. Incubate with rotation for 30 minutes.
3. Wash the beads four times with buffer A.
4. Incubate the beads for 10 minutes with 0.6 ml of 5M guanidine HCl, 30% isopropanol, 20 mM HEPES, 5 mM EDTA pH 7.5 (Elution).
5. Purify supernatant over a QIAquick spin column (Qiagen, cat #28104).

5. qPCR

The presence of DNA regions of interest in the material retained by the H6-GST-MBD fusion protein can be determined by end-point or by qPCR. Alternatively, the material can be analysed following amplification and labelling on high density oligonucleotide arrays. Use appropriate amounts of input material to quantify the fraction of methylated DNA recovered by this procedure. The initial unbound material contains unmethylated DNA and may also be useful for experimental analysis.

Results of QC on the H6-GST-MBD fusion protein



The Diagenode H6-GST-MBD fusion protein (cat# mbd-001-100) contains two tags: the GST which can bind to GSH and the His6-tag which can bind to nickel. A cross titration of BioGAM1 (oligonucleotide) and the MBD fusion protein was performed using serial dilutions in an alpha-screen assay. Results are shown in the two Figures above. The Figures show the interaction between BioGAM1 and the H6-GST-MBD fusion protein, using the GSH-Acceptor beads (left side). Interaction between BioGAM1 and the H6-GST-MBD fusion protein is also seen using the Nickel-Acceptor beads (right side).

In short:

MATERIAL

1. Assay buffer: 10 mM Tris pH 8.0, 3 mM MgCl₂, 100 mM NaCl, 0.01% Tween
2. Final concentration of Alpha screen beads: GSH-Acceptor: 4 µg/ml; Streptavidin - Donor: 4 µg/ml; Nickel-Acceptor: 4 µg/ml; Streptavidin-Donor: 4 µg/ml.
3. Work with 15 µl/well per reaction.
4. Work with 1:3 serial dilutions of oligo (from 100 to 0.05 nM/reaction) e.g. in 96-well plate: use wells A to H.
5. Work with 1:3 serial dilutions of H6-GST-MBD fusion protein (from 1,000 to 0.46 nM/reaction) e.g. in 96-well plate: use wells 1 to 8.

PROTOCOL

1. Prepare serial dilutions of the H6-GST-MBD fusion protein (to be diluted - in 8 tubes (1 to 8) - eight 1:3 serial dilutions: from 1,000 nM per assay down to 0.46 nM per assay) as described here after.
 - Before starting the serial dilution, you need 225 μ l of H6-GST-MBD fusion protein at 3,000 nM (add 2.5 μ l MBD fusion protein (cat# mbd-001-100) in 222.5 μ l of assay buffer).
 - Add 150 μ l of assay buffer in 8 tubes (tubes 1 to 8). From the 225 μ l of H6-GST-MBD fusion protein (tube above), transfer 75 μ l to the first tube (tube 1), mix and perform the next transfer similarly from tube 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7 and 7 to 8.
2. Prepare serial dilutions of the BIO-GAM1 (to be diluted - in 8 tubes (A to H)- eight 1:3 serial dilutions: from 100 nM per assay down to 0.05 nM per assay) as described here after.
 - Before starting the serial dilutions, you need 275 μ l of oligo at 300 nM (e.g., use 3.75 μ l in 372 μ l of assay buffer).
 - Add 250 μ l of assay buffer in 8 tubes (tubes A to H). From the 375 μ l of oligo (tube above), transfer 75 μ l to the first tube (tube A), mix and perform the transfer similarly from tube A to B, B to C, C to D, D to E, E to F, F to G and G to H.
3. In a Proxiplate plate: transfer each diluted methylated oligo (5 μ l/assay or well) and diluted H6-GST-MBD (5 μ l/assay or well), respectively, starting from highest dilutions: DNA (A-H) and H6-GST-MBD (1-8). The DNA transferred is serially diluted from upper to lower rows (A to B, to C, to D, to E, to F, to G and to H). The MBD fusion protein is serially diluted from lane A to H (left to right).
4. Incubate for 120 minutes at RT in the dark (10 μ l final per well).
5. Add the alpha-bead mix (5 μ l of 3x/assay) (15 μ l final per well).
6. Incubate 60 minutes at RT in the dark.
7. Read in envision.