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A Hologic Company

TECHNICAL DATASHEET

MethylCap protein

Cat. No. C02020012

Format: 100 µg/ 25 µl

Concentration: 4 mg/ml; MM 36.657 g/mol

Product description

This MethylCap protein (Cat. No. C02020012) has been extensively validated for specific isolation of DNA containing methylated CpGs. 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. A single fully methylated CpG is sufficient for the interaction between the MethylCap protein and methylated DNA

Format

Purified over a nickel affinity matrix. Supplied in solution in PBS containing 10% glycerol. 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 DNA. Electrophoresis on SDS-PAGE indicates that the protein is essentially homogeneous.

Storage conditions

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.

Reference paper

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.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode LLC. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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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. DNA preparation for MethylCap

1. Use the GenDNA module (Cat. No. mc-magme-003) from Diagenode and resuspend the pellet of DNA at ~1 mg/ml in GenDNA TE until dissolved.
   - From 1-1.5 million cells, ~8 to 12 µg of DNA can be expected (in a volume of 8 to 12 µl).
   - From 3 million cells, ~20 to 30 µg of DNA can be expected (in a volume of 20 to 30 µl).
   - From 10 million cells, ~50 to 100 µg of DNA can be expected (in a volume of 200 to 300 µl).
2. Measure the DNA concentration.
   - If possible, it is recommended to get at least 30 µg of DNA (when enough material is available) to be able to work with 30 µg of DNA: see 2/ DNA shearing protocol).
3. Run samples in a 1% agarose gel along with DNA size marker to visualize the DNA preparation efficiency.

2. Bioruptor-DNA Shearing Protocol

1. In a 1.5 ml tube, dissolve the DNA samples in GenDNA TE to reach 0.1 µg/µl.
2. Use a final volume of 300 µl of DNA samples in 1.5 ml tubes.
3. Shear the DNA using the Bioruptor® (www.diagenode.com).
   - power: High
   - cycles: [15 seconds "ON" & 15 seconds "OFF"]
   - total time: 10 minutes

Sheared DNA can be analysed on agarose gel to visualize the size of the sheared DNA. The expected size of the DNA fragments is 300-500 bp.

3. Binding

1. Take 5 µg of sheared DNA into a 1.5 ml tube and add 2 µg of MethylCap fusion protein.
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. No. 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) and recover the supernatant.
5. Purify supernatant over a QIAquick spin column (Qiagen, Cat. No. 28104).

5. qPCR analysis

The presence of DNA regions of interest in the material retained by the MethylCap 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.