MethylAffinity™ Methylated DNA Enrichment Kit
For rapid purification and enrichment of methylated DNA

Cat.No.MAK-GCM-30 (30 reactions)

User Manual

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USER MANUAL

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I. Introduction

DNA methylation in mammals is involved in many cellular processes and is responsible for gene silencing by recruitment of transcriptional repression complexes. It plays a critical role in development. However, massive methylation of the promoter regions has been frequently observed in cancer, which can cause transcription repression of tumor suppressor and DNA repair genes.

MethylAffinity™ Methylated DNA Enrichment Kit provides a GCM™-bead-based method for quick enrichment of methylated DNA fragments from whole genome[1]. The enriched sample improves the performance of downstream analysis, such as RT-PCR, microarray and sequencing, for methylation status and location studies.

GCM™ recombinant protein is designed based on the functional domain of methyl binding domain (MBD) proteins[2]. It binds specifically to double-stranded DNA (e.g.genomic DNA) containing methylated CpG dinucleotides, unlike methylated DNA antibodies which can only bind to single-stranded DNA.

Compared to wild-type MBD proteins, GCM™ has much stronger affinity for methylated DNA and minimal affinity for non-methylated DNA, which makes the binding more specific. The affinity for GCM™ is proportional to the density of methylated CpG dinucleotides contained in the DNA fragment.

II. Advantages and Protocol Overview

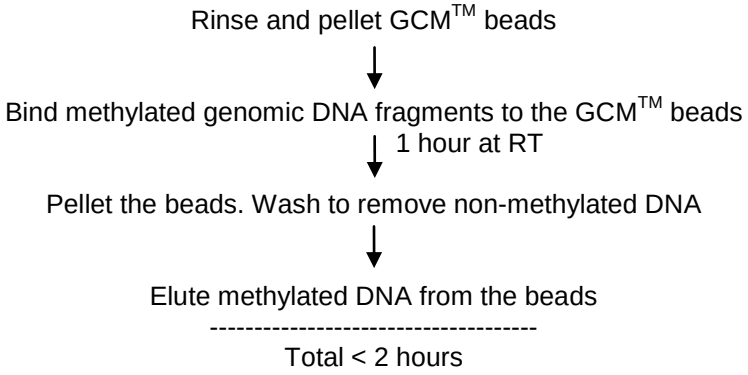
Fewer steps and less time
In MethylAffinity™ Methylated DNA Enrichment Kit, GCM™ is provided as immobilized GCM™ magenta beads. GCM™ was cross-linked to GeneCopoeia's proprietary magenta beads through strong non-covalent binding. The immobilized GCM™ beads significantly simplify the protocol. Starting from fragmented DNA samples, the whole procedure can be completed in less than 2 hours (see Protocol Overview).

Highly Sensitive
The genetically engineered GCM™ has much higher affinity than the wild type MBD. The GCM™ beads are able to isolate DNA fragments which contain only a few of methylated CpG dinucleotides and can be used to enrich methylated DNA from nanograms of genomic DNA sample.

Easy to handle
The magenta color of the GCM™ beads makes the pellet very easy to spot, which greatly reduces the risk of incidental beads loss.

Consistency
All kit components are produced using highest-quality reagents. The kits are strictly quality controlled to ensure batch-to-batch consistency.

Protocol Overview



III. Kit Components and Storage

Sufficient kit components are provided for enriching methylated DNA from up to 30 µg of fragmented genomic DNA.

Contents	Amounts (30 rxns)	Shipping temperature	Storage temperature
GCM™ beads (25% suspension)†	600 µl	Ice pack	-20°C Stable for at least 12 months
Binding Buffer (contains 300 mM NaCl)	35 ml	Ice pack	4°C Stable for at least 12 months
Elution Buffer (contains 1 M NaCl)	5 ml	Ice pack	4°C Stable for at least 12 months
Low salt Buffer (contains no NaCl)	10 ml	Ice pack	4°C Stable for at least 12 months
High salt Buffer (contains 2 M NaCl)	10 ml	Ice pack	4°C Stable for at least 12 months
pUC19 DNA (0.5 µg/µl, sonicated)	150 µl	Ice pack	-20°C Stable for at least 12 months
Mouse genomic DNA[3], sonicated (100 ng/µl)	15 µl*	Ice pack	-20°C Stable for at least 12 months
Beta actin promoter primer set for negative control[3] (2 µM)	30 µl*	Ice pack	-20°C Stable for at least 12 months
GAPDH promoter primer set for negative control[3] (2 µM)	30 µl*	Ice pack	-20°C Stable for at least 12 months
IAP primer set for positive control[3] (2 µM)	30 µl*	Ice pack	-20°C Stable for at least 12 months
DNA fragmentation enzyme (2,000 units/ml)	30 µl	Ice pack	-20°C Stable for at least 12 months
DNA fragmentation buffer (10×)	150 µl	Ice pack	-20°C Stable for at least 12 months
DNA LoBind tubes	30	Ice pack	Room temperature

Note:
†GCM™ beads are supplied as 25% (v/v) suspension in a buffer containing 50% (v/v) glycerol. Remove only the amount of beads required shortly before use.
*Sufficient mouse genomic DNA and control primer sets are provided for 15 control reactions.

Materials required but not supplied

Rotisserie Shaker
DNase-free ultrapure water
3.0 M sodium acetate, pH 5.2
100% ethanol
75% ethanol
TE buffer: 10 mM Tris-Cl (pH 7.5), 1 mM EDTA
Reagents for PCR assays

IV. DNA preparation and fragmentation

1. Isolate genomic DNA using an established protocol. Determine the DNA concentration by UV absorbance at 260nm. Check the DNA quality by agarose gel electrophoresis and ratio of OD260nm/OD280nm.

2. Fragment the genomic DNA using either sonication or restriction enzyme digestion. In either case, make sure that the genomic DNA is fragmented to 250-500 bp in length.

   The following protocol is the DNA fragmentation procedure using restriction enzyme digestion. The DNA fragmentation enzyme is a mix of restriction enzymes that cut DNA at non-CpG region.

   1) Prepare DNA restriction digestion reaction in a DNase-free tube as indicated below:

10×DNA fragmentation buffer	5.0 µl
Genomic DNA	up to 2 µg
DNA fragmentation enzyme	2.0 µl
Double-destilled H_2O to bring the final volume to	50 µl

   Tap the bottom of the tube gently to mix well. Spin the tube briefly.

   Note: Use more fragmentation enzyme if more genomic DNA is to be digested. 1µl of fragmentation enzyme is able to digest ~1 µg of genomic DNA.

   2) Incubate the tube at 37^{o}C for 2 hours or overnight.
   3) Check the completion of cleavage by agarose gel (2-3%) electrophoresis using a fraction of the digestion reaction.
   4) Incubate the tube at 70^{o}C for 20 minutes to inactivate enzymes.

   The fragmented genomic DNA samples can be stored at -20^{o}C or processed with GCM™ beads following the instruction in Section V.

V. Methylated DNA enrichment procedure

The following protocol is for enriching methylated DNA from **up to 1 µg** of fragmented genomic DNA samples. For DNA samples greater than 1 µg, scale up proportionally.

Basic protocol (one-step elution)

1. Rinse and pellet the GCM™ beads

1) Gently tap the GCM™ beads tube to resuspend the beads evenly.
2) Transfer 20 µl of beads suspension to a 1.5 ml DNase free tube.
3) Add 200 µl of Binding Buffer to the beads. Mix gently.
4) Spin the tube briefly to pellet the beads. Carefully aspirate and discard the supernatant.

2. Bind methylated DNA to the GCM™ beads

Sample tube:

1) Add fragmented genomic DNA (up to 1 µg) to the 1.5ml tube containing the GCM™ beads.
2) Then add 5 µl (2.5 µg) of pUC19 DNA to the tube.
3) Bring the final volume to 100 µl with Binding Buffer.

Control tube:

1) Add 1 µl mouse genomic DNA[3] (100 ng) provided in the kit to the 1.5ml tube containing the GCM™ beads.
2) Then add 5 µl (2.5 µg) of pUC19 DNA to the tube.
3) Bring the final volume to 100 µl with Binding Buffer.

Close the tubes tightly and rotate the tubes on a rotisserie shaker for 1 hour at room temperature. Alternatively, the tubes can be rotated overnight at 4°C.

3. Wash the beads

1) Spin the tubes at 1,000×g for 1 minute to pellet the beads.
2) Carefully transfer the supernatants to new tubes (**Note:** Save the supernatants here to make sure the binding reaction was performed correctly and the methylated DNA has been captured).
3) Wash the beads with 200 µl Binding Buffer for 4 times. Carefully aspirate and discard the supernatant completely.

4. One-step elution

1) Resuspend the beads with 25 µl Elution Buffer. Mix gently but thoroughly.
2) Incubate for 5 minutes at room temperature.
3) Spin the tubes at 1,000×g for 1 minute to pellet the beads.
4) Transfer the supernatants that contain eluted methylated DNA to new tubes.
5) Repeat the above elution process and pool the supernatants from this step and the previous step.

Note: For gradient elution, please see below.

Gradient elution protocol (Optional)

To study methylation status precisely, sometimes fractionation of methylated DNA fragments based on the density of methylated CpG dinucleotides is required. In such cases, gradient elution can be performed.

In the MethylAffinity™ Methylated DNA Enrichment Kit, besides the standard Elution Buffer, which contains 1 M NaCl, a Low Salt Buffer (containing no NaCl) and a High Salt Buffer (containing 2 M NaCl) are also provided. Gradient concentrations of NaCl (from 350 mM to 2 M) can be easily prepared by mixing the Low Salt and High Salt Buffers at different ratios.

Instead of performing standard elution at Step 4, elution buffers with gradient salt concentration from low to high can be used to elute and fractionate methylated DNA fragments based on the density of methylated CpGs.

Note:

The enriched methylated DNA can be used directly for quantitative PCR analysis or stored at -20°C for later use. The DNA can also be precipitated by adding 3 M sodium acetate (at 1/10th sample volume) and cold 100% ethanol (at 3-fold of sample volumes).

VI. Appendix

Positive and negative control PCR reaction conditions and primer sequences[3]

0.5 µl Template (enriched mouse genomic DNA)
0.25 µl Hot Start Taq polymerase (5U/µl)
5 µl 5x PCR reaction buffer
2 µl primer set 2µM
2 µl 2.5 mM dNTP mix
Add H_2O to bring the final volume to 25 µl

PCR cycling conditions
95°C 10 min. x 1
95°C 10 sec., 60°C 10 sec., 72°C 15 sec. x 35 cycles
72◦C 7 min. x 1

The sequences of Beta actin promoter primers (negative control):
F: 5'AGCCAACTTTACGCCTAGCGT3'
R: 5'TCTCAAGATGGACCTAATACGGC3'

The sequence of GAPDH promoter primers (negative control):
F: 5'CTCTGCTCCTCCCTGTTCC3'
R: 5'TCCCTAGACCCGTACAGTGC3'

The sequence of IAP primers (positive control):
F: 5'CTCCATGTGCTCTGCCTTCC3'
R: 5'CCCCGTCCCTTTTTTAGGAGA3'

VII. References

1. Cross, S.H., Charlton, J.A., Nan, X., and Bird, A.P. (1994). Purification of CpG islands using a methylated DNA binding column. Nature genetics 6, 236-244.
2. Fatemi, M., and Wade, P.A. (2006). MBD family proteins: reading the epigenetic code. Journal of cell science 119, 3033-3037.
3. Mohn, F., Weber, M., Schubeler, D., and Roloff, T.C. (2009). Methylated DNA Immunoprecipitation (MeDIP). Methods in molecular biology 507, 55-64

VI. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of the MethylAffinity™ Methylated DNA Enrichment Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

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GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be

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