MethylDetector™ Bisulfite Modification Kit

(version B)

Catalog No. 55001

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Overview

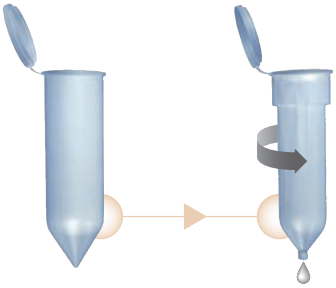
Active Motif's MethylDetector™ Bisulfite Modification Kit simplifies analysis of DNA methylation by providing optimized reagents for performing DNA conversion with bisulfite, plus time-saving DNA purification columns and positive control PCR primers to validate results.

DNA methylation is a naturally occurring event that affects cell function by altering gene expression. In methylation, a methyl group is added to the fifth-carbon of cytosine in a CpG dinucleotide by DNA methyltransferase. As aberrant methylation is prevalent in many human cancers, and because methylation is also involved in embryonic development and cell cycle regulation, much research depends on accurately quantifying DNA methylation. Many DNA methylation analysis methods begin by using bisulfite to convert unmethylated cytosines to uracils.[1,2] During conversion, methylated cytosines remain unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. A methylation profile of the sample can then be created by comparing the sequence of the converted DNA to untreated DNA. However, bisulfite conversion can be technically challenging, and it is desirable to confirm that the process was successful before costly analysis of samples. The MethylDetector Kit provides optimized conversion reagents, an easy-to-use protocol and positive control PCR primers that are specific for bisulfite-converted human DNA. Because these primers produce PCR product only if conversion has occurred, you can confirm the procedure worked before starting sequencing or other analysis methods.

In the MethylDetector method, DNA of interest is rapidly heat denatured in a thermocycler in the presence of the bisulfite conversion reagent. The temperature is then lowered and the conversion reaction is performed. Unlike other methods, MethylDetector does not require an initial alkaline denaturation step as the conversion reagent includes a DNA denaturant. After DNA conversion, the sample is added to the included DNA purification columns, and a simple, on-column desulfonation is performed. Ready-to-use DNA is then eluted from the columns. For your convenience, the included positive control PCR primers can be used to assess the success of the bisulfite conversion before DNA sequencing because the included primers only anneal to converted human DNA.

product	format	catalog no.
MethylDetector™	50 rxns	55001

Flow Chart of Process



Flow chart of the MethylDetector™ process.

In MethylDetector, DNA of interest is rapidly heat denatured in a thermocycler in the presence of the bisulfite conversion reagent. The temperature is then lowered and the conversion reaction is performed in the presence of a DNA denaturant. After DNA conversion, the sample is added to the included DNA purification columns, and a simple on-column desulfonation is performed. DNA is then eluted from the column and is ready for use in PCR.

Introduction

Over the last decade, the study of DNA methylation and its role in epigenetic cell signaling has grown rapidly[3-6]. Cellular methylation of CpG-dinucleotides, which occurs at the fifth position of the cytosine pyrimidine ring, is of particular interest.

Although CpG dinucleotides are generally methylated throughout the genome of normal somatic cells, CpG islands (clusters of CpG dinucleotides in gene regulatory regions) are usually unmethylated[7]. Aberrant hypermethylation of CpG islands and subsequent transcriptional repression is one of the earliest and most common somatic genome alterations in multiple human cancers[8, 9]. Somewhat paradoxically, a decrease in the total amount of cytosine methylation is observed in many neoplastic tissues, but the genome context of this hypomethylation has not been identified[10]. Aberrant methylation of CpG islands thus seems to be a tumor type-specific event[9, 11] and current efforts have concentrated on finding ways to exploit the diagnostic and therapeutic implications of these abnormalities[12, 13].

Traditional Methods to Study DNA Methylation

To date, there are several methods used for methylation analysis:

1. **Methylation-sensitive restriction enzyme analysis:** Isoschizomers of bacterial restriction endonucleases with different sensitivities for 5-methylcytosine can be used to determine the methylation status of specific CpG-dinucleotides[14]. Methylation-sensitive restriction

enzymes have several limitations including that methylation-sensitive restriction merely informs on the methylation status of the cytosine residues which are recognized by the restriction enzymes used.

2. **Bisulfite conversion:** Bisulfite conversion[15] consists of the treatment of double-stranded genomic DNA with sodium bisulfite, leading to deamination of unmethylated cytosines into uracil. PCR is then performed with primers that differentiate between methylated and unmethylated sequences. Bisulfite converted DNA can be sequenced in order to quantify the level of DNA methylation in the sample. However, bisulfite-based techniques do involve chemical treatments that can damage DNA and limit throughput. Additionally, PCR primer design becomes difficult due to reduction in genome complexity after bisulfite treatment.
3. **Methylated DNA Immunoprecipitation (MeDIP):** In this assay, an antibody specific for methylated cytosines (anti-5-methylcytosine antibody) is used to immunoprecipitate methylated DNA from genomic DNA fragmented by enzymatic digestion or sonication[16]. The resulting enrichment is usually analyzed by PCR based methods; thus MeDIP can be combined with DNA microarrays for genome-wide analysis of CpG methylation. However, this technique is relatively time-consuming, requires a large amount of fragmented DNA starting material and only works with denatured DNA.

Kit Performance and Benefits

The MethylDetector Kit is for research use only. Not for use in diagnostic procedures.

Sensitivity: MethylDetector can be performed on 200 pg - 2 µg of DNA.

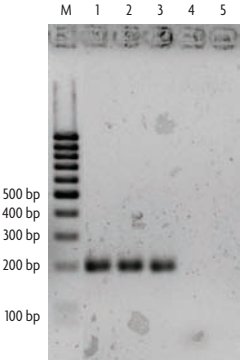


Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.

The MethylDetector DNA conversion method was performed in triplicate on human genomic DNA. The kit's conversion -specific positive control primers were used to perform PCR on the converted DNA (lanes 1-3) and on unconverted DNA (lane 5). Lane 4 is a no DNA control. The absence of PCR product in lane 5 demonstrates the conversion-specificity of the PCR primers while the PCR product in lanes 1-3 confirms that the DNA conversion was successful.

Kit Components and Storage

Kit components arrive at room temperature and can be stored at 4°C prior to first use. Then, we recommend storing each component at the temperatures listed in the table below:

Reagents	Quantity	Storage / Stability
Conversion Reagent	5 tubes	RT for 6 months
Denaturation Reagent	875 µl	RT for 6 months
Hydroquinone	5 x 100 µl	RT for 6 months
Buffer A	1.75 ml	RT for 6 months
Buffer B	1.1 ml	RT for 6 months
DNA Binding Buffer	2 x 15 ml	RT for 6 months
DNA Wash Buffer	25 ml	RT for 6 months
DNA Elution Buffer	2.5 ml	RT for 6 months
DNA purification columns	50 ea	RT for 6 months
DNA column collection tubes	50 ea	RT for 6 months
p16 Outer Primer Pair	200 µl	-20°C for 6 months
p16 Inner Primer Pair	200 µl	-20°C for 6 months
10X PCR Buffer	1.5 ml	-20°C for 6 months

Additional Materials Required

- 0.2 ml PCR tubes and caps
- 100% isopropanol
- Microcentrifuge tubes and microcentrifuge
- dNTP mixture (5 mM each)
- PCR cycler
- *Taq* polymerase (5 U/µl) (Example: New England Biolabs M0267L or GeneSpin STS-T1000)

Buffer Preparation and Recommendations

Preparation of Conversion Buffer

Five tubes of Conversion Reagent are provided in MethylDetector. Each vial is sufficient for performing 10 bisulfite conversions. Prepare the Conversion Buffer by resuspending one of the Conversion Reagent tubes with 700 µl dH_2O, 350 µl Buffer A and 175 µl Denaturation Reagent. Mix at room temperature for 10 minutes with intermittent vortexing.

NOTE: This is a saturated solution and it is normal for the Conversion Reagent to not dissolve completely. The Conversion Buffer is stable for 1 week at -20°C.

Preparation of Hydroquinone

Hydroquinone is an alkalizing agent and prevents DNA from strand breakage because of depurination. Each tube provided can be used for 10 conversion reactions. Prepare a working stock of Hydroquinone by resuspending one tube with 100 µl dH_2O. Keep mixture in dark and discard after use.

Preparation of Desulfonation Buffer

For each reaction, combine 22 µl Buffer B with 88 µl dH_2O and 110 µl 100% isopropanol. If this reagent will not be used immediately, it can be stored for up to one week at -20°C.

Positive control PCR primers

The MethylDetector's positive control PCR primers are specific for converted human DNA. These primers amplify a region of the p16 locus and demethylation of this locus has been shown to correlate with up-regulation of the p16INK4A gene in human gastric cancer cell lines[17]. This locus has an official symbol of CDKN2A (cyclin-dependent kinase inhibitor 2A) and is located on chromosome 9, location 9p21 in humans. Other aliases include HGNC:1787, ARF, CDK4I, CDKN2, CMM2, INK4, INK4a, MLM, MTS1, p16, p14, p14ARF, p16, p16INK4, p16INK4a, p19.

Design of PCR primers

PCR primer design is critical for successful analysis of converted DNA. There are several sites that offer information on designing methylation PCR primers. The MethPrimer website[18]: **http://www.urogene.org/methprimer/** is a free site that contains several useful tools for the design of bisulfite-conversion-based Methylation PCR Primers. In addition, PREMIER Biosoft International offers the Beacon Designer program **http://www.premierbiosoft.com/crm/jsp/com/pbi/crm/clientside/ProductList.jsp**. To ensure effective and specific amplification of the desired target, we recommend that nested PCR primers be used whenever possible.

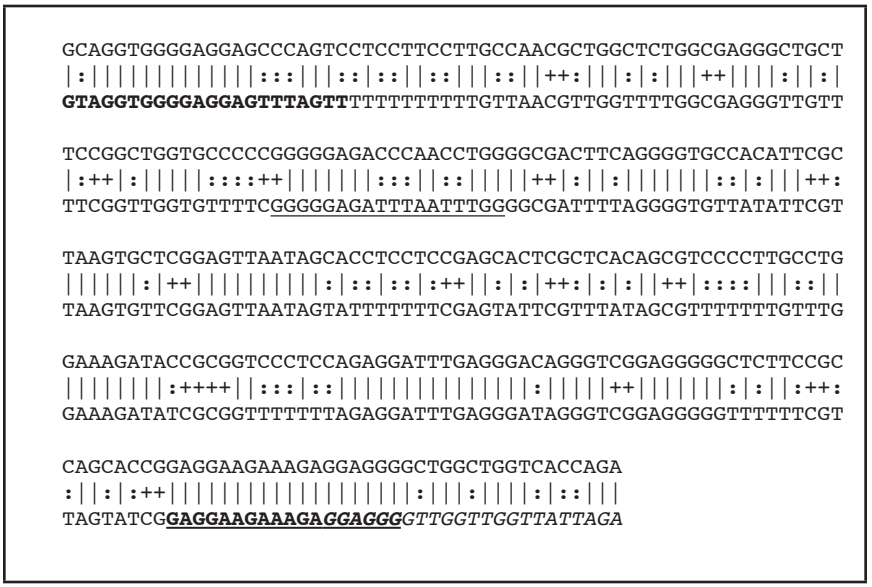


Figure 2: MethylDetector's PCR primers are specific for only converted DNA

The figure above depicts the region of the human p16 locus that is used as the positive control in MethylDetector. The top row is the original, unconverted sequence, while the lower row is the bisulfite modified sequence. For this display, it is assumed that all CpG sites are methylated. As depicted in the figure, the p16 outer primers and the p16 inner forward primer are conversion specific because they anneal with no mismatches to the converted sequence, while each primer has several mismatches when compared with the unconverted sequence. The p16 inner reverse primer matches 100% with both the converted and the unconverted DNA.

Legend

++ Indicates a CpG site, : Indicates Non-CpG 'C' converted to 'T'

The p16 outer forward primer is in bold. The p16 outer reverse primer anneals to the sequence and is in italics. The p16 inner forward primer is underlined and the p16 inner reverse primer anneals to the sequence and is bold and underlined. The p16 inner reverse primer and the p16 outer reverse primer overlap slightly.

The outer primer sequences are:
p16 Forward outer: GTAGGTGGGGAGGAGTTTAGTT
p16 Reverse outer: TCTAATAACCAACCAACCCCTCC
The outer pair flanks a 283-bp region of the p16 locus.

The Inner primer sequences are:
p16 Forward inner: GGGGGAGATTTAATTTGG
p16 Reverse inner: CCCTCCTCTTTCTTCCTC

MethylDetector Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

The amount of DNA used in MethylDetector should range from 200 pg to 2 µg, with 500 ng to 2 µg optimal. Please see the Appendix A: Troubleshooting section for suggestions when using less than 100 ng of DNA. In addition, the positive control PCR primers are designed to anneal to converted human DNA. If you plan to perform the positive control reactions, it is recommended that you perform the conversion reaction with 500 ng of human DNA.

Step 1: Conversion Reaction

1. Prepare Conversion Buffer and Hydroquinone as described in the Buffer Preparation section on page 4.
2. Set up a conversion reaction for each sample by adding the reagents in the order listed below into PCR tubes. Mix well by pipetting. The final volume should be 140 µl. If using less than 13 µl of DNA, adjust the volume with water to a final volume of 140 µl. Keep hydroquinone away from light.

Reagent	Quantity
DNA	13 µl
dH2O	X µl
Hydroquinone	7 µl
Conversion Buffer	120 µl
Total Volume	**140 µl**

3. Place tubes in thermocycler and set the program to start with an initial melt at 94°C for 3 minutes, then a 50 °C conversion for 5 hours, followed by a hold at 4°C. To prevent evaporation of samples, use the heated lid or overlay reactions with mineral oil.
4. Remove tubes and continue with Step 2 below. The protocol can also be stopped here and DNA samples can be kept at 4°C in the dark for up to 5 days.

Step 2: On-column desulfonation and DNA purification

1. Prepare Desulfonation Buffer as described in the Buffer Preparation section on page 4.
2. For each conversion reaction, aliquot 500 µl DNA Binding Buffer into a 1.5 ml microcentrifuge tube. Add the entire conversion reaction mixture to the DNA Binding Buffer in the tube and vortex.
3. Remove the desired number of DNA purification columns and place each one in a collection tube.
4. Pipet each DNA Binding Buffer/conversion reaction mixture into one of the DNA Purification columns and spin at 10,000 rpm for 30 seconds in a microcentrifuge. Empty the collection tube and place the column back on the collection tube.

5. Add 200 µl DNA Wash Buffer to each column and spin at 10,000 rpm for 30 seconds in a microcentrifuge.
6. Add 200 µl Desulfonation Buffer to each column and incubate 20 minutes at room temperature. Spin at 10,000 rpm for 30 seconds in a microcentrifuge. Empty the collection tube and place the column back on the collection tube.
7. Add 200 µl DNA Wash Buffer to each column and spin at 10,000 rpm for 30 seconds in a microcentrifuge. Remove the column and place in a new 1.5 ml microcentrifuge tube.
8. Add 50 µl DNA Elution Buffer directly to the filter of the column.
9. Incubate 3 minutes at room temperature. Spin at 10,000 rpm for 30 seconds in a microcentrifuge. The eluate will contain the converted DNA.
10. The eluted DNA is now ready for analysis by using methylation-specific PCR or PCR amplification followed by sequencing.

OPTIONAL - Use of Positive Control p16 Primers to confirm conversion

Step 3: Initial PCR using p16 Outer primers

Set up a PCR reaction by using the p16 Outer Primer Pair. Three reactions are performed: one with converted DNA as template, one with dH_2O as template (the no DNA control) and one with 40 ng unconverted DNA as template.

1. Label PCR tubes 1-3 and place in a PCR tube rack on ice.
2. Make a 10 ng/µl dilution of uncut, unconverted human genomic DNA (the same DNA that was used for the conversion reaction).
3. Prepare p16 outer PCR cocktail in 1.5 ml microcentrifuge tubes as described below. DNA templates will be added later directly to the PCR tubes. Combine components on ice and mix by vortexing. Four reactions are prepared to account for any loss during pipetting.

Reagent	One reaction	4 reactions
dH_2O	19.3 µl	77.2 µl
p16 Outer Primer Pair	3.0 µl	12 µl
dNTP mixture (10 mM each dNTP)	0.5 µl	2.0 µl
10X PCR Buffer	3.0 µl	12 µl
Taq (5 U/µl)	0.2 µl	0.8 µl
Total Volume (Not including DNA template)	26 µl	104 µl

Note: 10X PCR buffer provided in the kit is for your convenience. If preferred, use the 10X PCR buffer provided by the manufacturer of the *Taq* polymerase enzyme being used in the PCR reaction.

4. Aliquot 26 µl of this PCR cocktail to the PCR tubes 1-3 on ice.
5. Add template DNA to the PCR tubes as indicated below and mix. Ensure that the PCR reaction is on the bottom of the tube and centrifuge tubes if necessary.

PCR rxn #	PCR cocktail	DNA template	dH_2O
1	26 µl	4 µl converted DNA	0
2	26 µl	0	4 µl
3	26 µl	4 µl 10 ng/µl unconverted DNA	0

6. Place tubes in PCR cycler and program the thermocycler as below. (To prevent evaporation of samples, use the heated lid or overlay reactions with mineral oil.)

   94°C for 3 minutes
   (94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds) for 20 cycles
   72°C for 4 minutes
   Hold at 4°C

Step 4: Nested PCR using p16 Inner primers

1. After the initial PCR is complete, label three PCR tubes 1-3 and place on ice.
2. Prepare p16 inner PCR cocktail in 1.5 ml microcentrifuge tubes as below. DNA templates will be added later directly to the PCR tubes. Combine components on ice and mix by vortexing. Four reactions are prepared to account for any loss during pipetting.

Reagent	One reaction	4 reactions
dH_2O	19.3 µl	77.2 µl
p16 Inner Primer Pair	3.0 µl	12 µl
dNTP mixture (10 mM each dNTP)	0.5 µl	2.0 µl
10X PCR Buffer	3.0 µl	12 µl
Taq (5 U/µl)	0.2 µl	0.8 µl
Total Volume (Not including DNA template)	26 µl	104 µl

3. Aliquot 26 µl of this PCR cocktail to the PCR tubes 1-3 on ice.
4. Add 4 µl of the Outer PCR DNA to the appropriate Inner PCR tube and mix as in table below. Ensure that the PCR reaction is on the bottom of the tube and centrifuge tubes if necessary.

PCR rxn #	PCR Cocktail	µl for 3 ChIP rxns
1	26 µl	4 µl Outer PCR1
2	26 µl	4 µl Outer PCR2
3	26 µl	4 µl Outer PCR3

5. Place tubes in PCR cycler and program the thermocycler as below. (To prevent evaporation of samples, use the heated lid or overlay reactions with mineral oil.)

   94°C for 3 minutes
   (94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds) for 30 cycles
   72°C for 10 minutes
   Hold at 4°C

Step 5: Agarose Gel Analysis of PCR products

1. Pour a 2.5% low molecular weight agarose gel in 1X TAE.
2. Add 10 µl from each of the p16 inner PCRs to 2 µl 6X DNA loading dye and load sample on a gel. Use a 100 or 50 bp ladder. Run the gel until the marker is near the bottom of the gel and the markers are well separated. Only the lane containing the converted DNA should show a strong band at 190 bp. See sample data on page 3.

Step 6: DNA clean up

Prior to cloning it is necessary to clean up the DNA. Use the following protocol to perform a phenol/chloroform extraction followed by ethanol precipitation. Alternatively, DNA can be purified using columns such as QIAquick PCR purification kit (Qiagen part no. 28104). Elute in 30-50 µl volume. If more than one band is amplified by the nested PCR reaction in Step 4, the DNA band of the expected size should be gel-purified using a gel extraction kit such as QIAEX II gel extraction kit (Qiagen part no. 20021).

1. To the remaining PCR reaction from Step 4, add the necessary amount of sterile water to make the total volume 100 µl.
1. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v/v) to the sample.
2. Vortex the tube at maximum speed for 15 seconds.
3. Centrifuge the tube for 5 minutes at 12,000 x *g* at room temperature.
4. Carefully transfer the top aqueous phase to clean microcentrifuge tube without collecting any of the lower organic phase or precipitate that may occur between the phases.
5. To each sample add:

   1 µl Glycogen (20 ng/µl)

   1/10 sample volume of 3 M sodium acetate pH 5.5

   2.5 sample volumes of 100% ethanol
6. Mix well and incubate at -80°C for at least 2 hours.
7. Centrifuge the tube for 20 minutes at 12,000 x *g*, 4°C.
8. Carefully discard the supernatant without disturbing the pellet.
9. Add 500 µl of cold 70% ethanol.
10. Centrifuge the tube for 10 minutes at 12,000 x *g*, 4°C.

11. Carefully discard the supernatant without disturbing the pellet.
12. Air-dry the pellet for 5-10 minutes (do not completely dry the pellet).
13. Resuspend the DNA pellet in 30 µl sterile DNase-free water.
14. This eluted DNA can be used immediately in PCR or stored at -20°C. If the DNA is stored at -20°C, it will need to be reheated at 37°C for 10 minutes prior to use to ensure DNA does not stick to the walls of the tube.

Step 7: DNA cloning

1. The cleaned PCR product can be cloned into a DNA vector of choice according to the instructions of the manufacturer. If possible, plasmids containing the *Lac*Z gene for blue/white screening are recommended.
2. Transform competent bacterial cells, such as RapidTrans™ TAM 1 (Active Motif Cat. No. 11096), with 2 µl of the ligation reaction. Follow the appropriate protocol for the competent cells used. The RapidTrans™ TAM 1 chemically competent cell protocol is listed below as an example.
    a. Remove required number of transformation reaction tubes from –80°C storage and place on ice to thaw.
    b. Add 1-5 µl of plasmid DNA/ligation to thawed cells. Mix by tapping tubes gently and replace on ice immediately. Do not mix by vortexing or pipetting. Do not add more than 5 µl (10% of competent cell volume) to reaction.
    c. Incubate transformation reactions on ice for 30 minutes.
    d. Heat-shock the tubes by immersing in a 42°C water bath for exactly 30 seconds.
    e. Replace transformation reactions on ice for 2 minutes.
    f. Aseptically add 250 µl SOC medium to each reaction.
    g. Incubate tubes at 37°C for 1 hour with shaking at 225-250 rpm.
    h. Using a sterile spreader, plate out 20-200 µl of each transformation on pre-warmed LB agar plates. If using blue/white screening, ensure that the selective plate contains X-Gal. For preparation of X-gal Stock solution and plates see note below.

    Note: Make a 40 mg/ml solution of X-gal in dimethylformamide (DMF). Use a glass or polypropylene tube and wrap in aluminum foil to prevent damage by light. Store at –20°C. Plate 40 µl of X-gal stock solution on top of the LB agar and let stand for approximately 1 hour. Alternatively, add the X-gal to the molten LB agar prior to pouring plates.

    i. Allow plates to completely absorb any excess media.
    j. Incubate inverted plates overnight at 37°C.
3. Select 10 colonies for screening. Select white colonies if using blue/white screening as the expression of *Lac*Z has been interrupted with the insertion of the ligation product.

4. Isolate plasmid DNA of the 10 selected colonies using any commercially available kit or published protocol.
5. Analyze the isolated DNA using restriction enzyme analysis to confirm the presence of the correct insert.
6. Submit DNA from the positive clones for sequencing. Use forward and reverse primers based on the DNA vector used for cloning. It is recommended to submit 10 colonies for sequencing to ensure representative results. It is also necessary to submit unconverted DNA for sequencing comparison.

Step 8: Analyzing the sequencing results

1. If the CpG dinucleotide was methylated in the original DNA, the sequencing read will show a CG at that location. If the CpG dinucleotide was unmethylated in the original DNA, the bisulfite conversion will have converted the cytosine to uracil and the sequencing read will show a TG at that location.
2. Compare the bisulfite converted sequence data against the unconverted DNA sequence.
3. Quantify the number of methylation sites for the region of interest to determine the percentage of methylation.

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Notes: The polymerase chain reaction (PCR) process for amplifying nucleic acid is covered by U.S. Patent Nos. 4,683,195 and 4,683,202 assigned to Hoffmann-La Roche. Patents pending in other countries.

Use of methylation-specific PCR (MSP) is protected by U.S. Patent Nos. 5,786,146, 6,017,704, 6,200,756 & 6,265,171 and International patent WO97/46705. No license under these patents to use the MSP process is conveyed to the purchaser by purchasing this product.

Appendix

Section A. Troubleshooting Guide

Problem/question	Recommendation
Using less than 100 ng of DNA as starting material	When converting a small amount of DNA, include 250 ng Salmon Sperm DNA as a carrier DNA in the conversion reaction to minimize loss. Use nested PCR primers if possible.
Starting DNA volume is greater than 13 µl	DNA volumes up to 20 µl can be used without affecting the protocol
Conversion time	A 5 hour incubation at 50°C is normally sufficient to efficiently convert DNA samples. However, DNA sequences that are GC-rich and/or contain extensive secondary structures may require a 9 hour conversion at 50°C. If noticing a loss of DNA after the conversion reaction, the conversion time can be reduced to as little as 3 hours.
Can I store my conversion reaction samples for longer than 5 days before performing the DNA purification and desulfonation steps?	The DNA is recommended to be stored at 4°C for 5 days. If longer storage is needed, store samples at -20°C. As precipitates can form during -20°C, long term storage is not recommended.
Storage of converted DNA	Once DNA is prepared using MethylDetector, samples may be stored at -20°C prior to PCR analysis. However, we do recommend heating the frozen material to 37°C for 10 minutes before use in PCR, as heat-treatment releases any DNA bound to the tube during storage.

Section B. Related Products

DNA Methylation	Format	Catalog No.
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003

Antibodies	Application	Format	Catalog No.
5-Hydroxymethylcytidine pAb	DB	100 µl	39769
5-Methylcytidine mAb	FACS, IHC, IP	50 µg	39649
CGBP rabbit pAb	WB	200 µl	39203
DNMT1 mouse mAb	ChIP, IHC, IP, WB	100 µg	39204
DNMT2 rabbit pAb	WB	100 µg	39205
DNMT3A mouse mAb	ChIP, IF, IHC, WB	100 µg	39206
DNMT3B mouse mAb	ChIP, IF, IP, WB	100 µg	39207
Kaiso mouse mAb	WB	200 µg	39365
MBD1 mouse mAb	WB	100 µg	39215
MBD2 rabbit pAb	WB	200 µl	39547
MBD3 mouse mAb	WB	100 µg	39216
MBD4 rabbit pAb	WB	100 µg	39217
MeCP2 rabbit pAb	WB	100 µg	39218
Ubiquitin mouse mAb	IF, IHC, IP, WB	100 µg	39741
Uhrf1 rabbit pAb	WB	200 µl	39625

Chromatin Immunoprecipitation	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009

ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
Bridging Antibody for Mouse IgG	500 µg	53017

Recombinant Methylated Histones	**Format**	**Catalog No.**
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Histone Purification & Chromatin Assembly	**Format**	**Catalog No.**
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	10 rxns	40026
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	**Format**	**Catalog No.**
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Co-Immunoprecipitation	**Format**	**Catalog No.**
Universal Magnetic Co-IP Kit	25 rxns	54002
Nuclear Complex Co-IP Kit	50 rxns	54001

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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