ab117133 – Methylated DNA Immunoprecipitation (MeDIP) Kit

Instructions for Use

For successful capture of methylated DNA from a DNA sample

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

A core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation, where methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Region-specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. Global DNA hypomethylation is likely caused by methyl-deficiency due to variety of environmental influences. It has been demonstrated that alterations in DNA methylation are associated with many diseases, and especially with cancer. Highly specific capture of methylated DNA should provide an advantage for convenient and comprehensive identification of methylation status of normal and diseased cells, such as cancer cells, that may lead to the development of new diagnostic and therapeutic methods in cancer. Several methods have been used for enriching methylated DNA such as agarose beads-based methylated DNA capture. How-ever, these methods so far are considerably time consuming, labor intensive, and have low throughput.

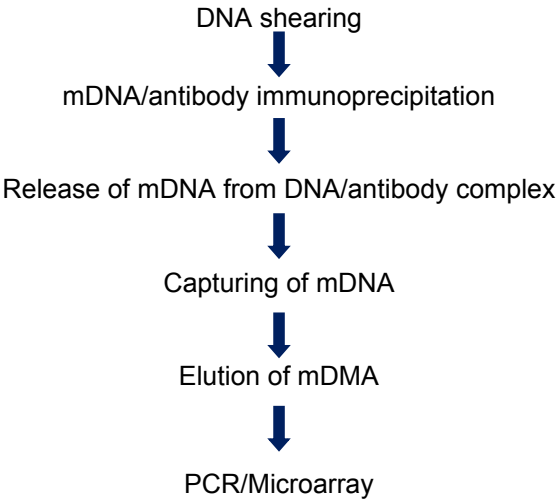
ab117133 uses a proprietary and unique procedure and compositions to enrich methylated DNA. In the assay, an antibody specific to methyl cytosine is used to capture methylated genomic DNA. The enriched methylated fractions can then be used for a standard DNA detection.

This kit has the following features:

- Highly efficient enrichment of methylated DNA: > 98%
- The fastest procedure available, which can be finished within 3 hours
- Strip microplate format makes the assay flexible: manual or high throughput
- Columns for DNA purification are included: save time and reduce labor
- Compatible with all DNA amplification-based approaches
- Simple, reliable, and consistent modification conditions

The Methylated DNA Immunoprecipitation (MeDIP) Kit - DNA contains all reagents required for carrying out a successful capture of methylated DNA from a DNA sample. Particularly, this kit includes a ChIP-grade 5-methylcytosine antibody and a negative control normal mouse IgG. DNA is sheared, added into the microwell, and captured by the antibody. DNA is released from the antibody-captured methylated DNA complex, and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

Avoid repeated thawing and re-freezing of temperature sensitive components. It is recommended that you aliquot accordingly ahead of time.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

Spin small vials after thawing and prior to use.

5. MATERIALS SUPPLIED

Item	24 Tests	48 Tests	Storage Condition (Before Preparation)
Antibody Buffer	8 mL	16 mL	4°C
Reaction Buffer	4 mL	8 mL	RT
Wash Buffer	16 mL	2 x 16 mL	4°C
DNA Release Buffer	2 mL	4 mL	RT
Binding Buffer	5 mL	8 mL	RT
Elution Buffer	0.6 mL	1.2 mL	RT
Normal Mouse IgG (1 mg/mL)	10 µL	20 µL	4°C
Anti-5-Methylcytosine (1 mg/mL)	25 µL	50 µL	4°C
Proteinase K (10 mg/mL)	25 µL	50 µL	4°C
8-Well Assay Strips (with Frame)	3	6	4°C
8-Well Strip Caps	3	6	RT
F-Spin Column	30	50	RT
F-Collection Tube	30	50	RT

*Spin the solution down to the bottom after thawing, prior to use.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Variable temperature waterbath
- Vortex mixer
- Desktop centrifuge (up to 14,000 rpm)
- Sonicator
- Orbital shaker
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- 15 mL conical tube
- 1 X TE buffer (pH 8.0)
- 90% Ethanol
- 100% Ethanol

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

DNA Release Buffer
Add 1 µL of Proteinase K to each 60 µL of DNA Release Buffer and mix.

All other solutions are supplied ready to use.

10. SAMPLE PREPARATION

Prepare DNA from cells of interest use a suitable method of extraction which should be determined by the user.

11. ASSAY PROCEDURE

11.1 Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C).

11.2 Add 100 µL of Antibody Buffer to each well and then add the antibodies: 1 µL of Normal Mouse IgG as the negative control, and 1 µL of Anti-5-Methylcytosine for the sample. Cover the strip wells with Parafilm M and incubate at room temperature for 60 minutes. Meanwhile, prepare the fragmented DNA as described in the following steps.

11.3 Add 0.5-1 µg of DNA to each 100 µL of Reaction Buffer (500 µL maximum for each 1.5 mL vial) and shear DNA by sonication. Usually, sonicate 3 pulses of 10-12 seconds each at level 2 using a Branson Microtip probe, followed by 30-40 seconds rest on ice between each pulse. (The conditions of DNA shearing can be optimized based on sonicator equipment. If desired, remove 5 µL of the sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp.)

11.4 Incubate sonicated DNA at 95°C for 2 minutes and immediately place on ice.

11.5 Remove 5 µL of the sonicated DNA solution to a 0.5 ml vial. Label the vial as "input DNA" and place on ice.

11.6 Remove the incubated antibody solution and wash the strip wells one time with 150 µL of Antibody Buffer and one time with 150 µL of Wash Buffer by pipetting in and out.

11.7 Add 100 µL of the sonicated DNA solution to each well. Cover the strip wells with Parafilm M and incubate at room temperature for 90-120 minutes on an orbital shaker (50-100 rpm).

11.8 Remove supernatant. Wash the wells three times with 150 µL of wash buffer.

11.9 Add 60 µL of DNA Release Buffer containing Proteinase K to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65ºC in a waterbath for 60 minutes.

11.10 Place a spin column into a 2 mL collection tube. Add 100 µL of Binding Buffer to the column. Add 180 µL of 100% ethanol to the samples and mix. Transfer the mixed solution to the column containing Binding Buffer. Centrifuge at 12,000 rpm for 20 seconds.

11.11 Remove the column and discard the flowthrough. Replace column to the collection tube. Add 200 µL of 90% Ethanol to the column and centrifuge at 12,000 rpm for 20 seconds.

11.12 Remove the column and discard the flowthrough. Replace column to the collection tube and wash the column again with 200 µL of 90% ethanol at 12,000 rpm for 35 seconds.

11.13 Place the column in a new 1.5 mL vial. Add 20 µL of Elution Buffer directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

Methylated DNA is now ready for use or storage at -20°C.

12. ANALYSIS

The user should determine the Quality of the Methylated DNA using an appropriate method.

For PCR positive control (methylation) and negative control (unmethylation), the primers for highly methylated sequences of H19ICR, LAP, or XIST and the primers for unmethylated β-actin or GAPDH sequence could be used, respectively.

For conventional PCR, the number of PCR cycles may need to be optimized for better PCR results

13. TROUBLESHOOTING

Problem	Cause	Solution
Little or No PCR Products	Insufficient starting DNA	Ensure the amount of starting DNA is sufficient (ex: 1 µg DNA/per reaction)
	Insufficient/too much sonication	Follow the protocol instruction for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication
	Incorrect temperature/insufficient time for DNA release	Follow the guidelines in the protocol for appropriate temperature and time
	Incorrect PCR conditions	Check if all PCR components are added. Increase amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction
	Incorrect or bad primers	Ensure the designed primers are specific to the target sequence
	The column is not washed with 90% ethanol	Ensure that wash solution is 90% ethanol
Little or No PCR Products	DNA is not completely passed through the filter	Increase centrifuge time to 1 minute at steps 11.10-11.13 of the protocol

Problem	Cause	Solution
Little or No Amplification Difference Between the Sample and the Negative Control	Insufficient wash at each wash step	Follow the protocol for appropriate wash
	Antibody is added into the well for the negative control by mistake	Ensure antibody is added into the correct well
	Too many PCR cycles	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured generally within the linear PCR amplification phase

14. NOTES

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