ab117135 – Methylated DNA Immunoprecipitation (MeDIP) ChIP Kit

Instructions for Use

For immunoprecipitating the methylated DNA from a broad range of species including human, rat, and mouse

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

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Table of Contents

1. BACKGROUND

The core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Region specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which plays an important role in the repression of gene transcription. Global DNA hypomethylation is likely caused by methyl-deficiency due to a variety of environmental influences. It has been demonstrated that alterations in DNA methylation are associated with many diseases, and especially with cancer.

Highly specific isolation of methylated DNA should provide an advantage for convenient and comprehensive identification of methylation status of normal and diseased cells, such as cancer cells, which may lead to the development of new diagnostic and therapeutic methods of cancer. Several methods have been used for enriching methylated DNA, including methyl-CpG binding domain (MBD) based methylated DNA affinity column and methylated DNA immunoprecipitation. However, these methods so far are considerably time consuming, labor intensive, have low throughput, and particularly, need purified DNA as starting material.

ab117135 uses a proprietary and unique procedure and composition to enrich methylated DNA. In the assay, an antibody specific to methyl cytosine is used to immunoprecipitate methylated genomic DNA. The immunoprecipitated methylated fractions can then be used for a standard DNA detection.

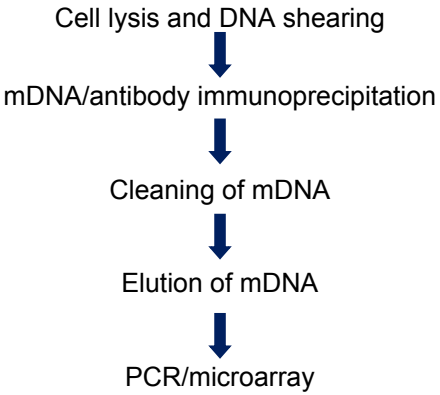
This kit has the following features:

- Directly immunoprecipitate the methylated fractions of DNA from cell lysates.
- Highly efficient enrichment of methylated DNA: > 95%.
- The fastest procedure available, which can be finished within 4 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 assay conditions.

ab117135 - contains all reagents required for carrying out a successful methylated DNA immunoprecipitation directly from mammalian cells. Particularly, this kit includes a ChIP-grade 5-methylcytosine antibody and a negative control normal mouse IgG. DNA in the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-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 Buffers contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	24 Tests	48 Tests	Storage Condition (Before Preparation)
Wash Buffer	28 mL	2 x 28 mL	RT
Antibody Buffer	15 mL	30 mL	RT
Pre-Lysis Buffer	4 mL	8 mL	RT
Lysis Buffer	4 mL	6 mL	RT
ChIP Dilution Buffer	4 mL	6 mL	RT
DNA Release Buffer	2 mL	2 x 2 mL	RT
Reverse Buffer	2 mL	2 x 2 mL	RT
Binding Buffer	5 mL	8 mL	RT
Elution Buffer	0.6 mL	1.2 mL	RT
Normal Mouse IgG (1mg/mL)*	10 µL	15 µL	4°C
Proteinase K (10mg/mL)*	25 µL	50 µL	4°C
Anti-5-Methylcytosine (1 mg/mL)*	25 µL	50 µL	4°C
8-Well Assay Strips (with 1 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
- 70% 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

Prepare 1X TE buffer (pH 8.0), 90% Ethanol and 70% Ethanol prior to starting the assay.

10. SAMPLE PREPARATION

10.1 Antibody Binding to Assay Strip Well

10.1.1 Predetermine the number of Assay Strip Wells required for your experiment. Carefully remove any unneeded strip wells from the plate frame and place the, back in the bag (seal the bag tightly and store at 4°C). Wash the strip wells once with 150 µL of 1X Wash Buffer.

10.1.2 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.

10.1.3 Cover the strip wells with Parafilm M and incubate at room temperature for 60 minutes. Meanwhile, prepare the cell extracts as described in the next steps.

10.2 Cell Collection and Cross-Linking

10.2.1 **For Monolayer or Adherent Cells:**

10.2.1.1 Grow cells (treated or untreated) to 80%-90% confluency. Remove culture medium and wash cells with PBS once. (At least 1 × 10^5 cells are required for each reaction).

Container	Cell number (x 10^5)
96-well plate	0.3-0.6/well
24-well plate	1-3/well
12-well plate	3-6/well
6-well plate	5-10/well
60 mm dish	20-30
100 mm dish	50-100

10.2.1.2 Add 20 µl of Lysis Buffer to each well for the 96-well plate; or 100 µl of Lysis Buffer for the 24-well plate; or 200 µl of Lysis Buffer for the 12-well plate; or 400 µl of Lysis Buffer for the 6-well plate; or 1.5 ml of Lysis buffer for a 100 mm plate. Incubate for 5 minutes at room temperature and pipette up and down several times to break cells.

10.2.2 **For Suspension Cells:**

10.2.2.1 Collect cells (treated or untreated) into a 15 mL conical tube. (2-5 x 10^5 cells are required for each reaction). Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.

10.2.2.2 Add Pre-Lysis Buffer to re-suspend the cell pellet (20 µL/1 x 10^5 cells). Transfer cell suspension to a 1.5 mL vial and incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge at 5000 rpm for 5 minutes. Discard the supernatant.

10.2.2.3 Add 10 µL of Lysis Buffer to each 1 x 10^5 cells.

11. ASSAY PROCEDURE

11.1 DNA Shearing

11.1.1 Transfer cell solution to a 1.5 mL vial (500 µL maximum for each vial) and incubate at room temperature for 10 minutes. Vortex vigorously for 10 seconds.

11.1.2 Shear DNA by sonication. Usually, sonicate 4 to 5 pulses of 10 to 12 seconds each at level 2 using a microtip probe, followed by 30 to 40 seconds rest on ice between each pulse. (The conditions of DNA shearing can be optimized based on cells and 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).

11.1.3 Pellet cell debris by centrifuging at 14,000 rpm for 10 minutes.

11.2 Methylated DNA Immunoprecipitation

11.2.1 Transfer clear supernatant to a new 1.5 mL vial (supernatant can be stored at -80°C at this step). Dilute required volume of supernatant with ChIP Dilution Buffer at a 1:1 ratio (e.g. add 100 µL of ChIP Dilution Buffer to 100 µL of supernatant).

11.2.2 Remove 5 µL of the diluted supernatant to a 0.5 mL vial. Label the vial as “input DNA,” and place on ice.

11.2.3 Remove the incubated antibody solution and wash the strip wells three times with 150 µL of Antibody Buffer by pipetting in and out.

11.2.4 Transfer 100 µL of the diluted supernatant to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature (22-25°C) for 60 to 90 minutes on a rocking platform (50-100 rpm).

11.2.5 Remove supernatant. Wash the wells six times with 150 µL of 1X Wash Buffer. Allow 2 minutes on a rocking platform (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 µLof 1X TE Buffer.

11.3 Methylated DNA Isolation/Purification

11.3.1 Add 1 µL of Proteinase K to each 40 µL of DNA Release Buffer and mix. Add 40 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 65°C in a waterbath for 15 minutes.

11.3.2 Add 40 µL of Reverse Buffer to the samples. Mix, and re-cover the wells with strip caps and incubate at 65°C in a water bath for 30 minutes. Also add 40 µL of Reverse Buffer to the vial containing supernatant, labeled as "input DNA." Mix and incubate at 65°C for 45 minutes.

11.3.3 Place a spin column into a 2 mL collection tube. Add 150 µL of Binding Buffer to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.

Note: *Always cap spin columns before placing them in the microcentrifuge.*

11.3.4 Add 200 µL of 70% Ethanol to the column, centrifuge at 12,000 rpm for 20 seconds. Remove the column from the collection tube and discard the flowthrough.

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

11.3.6 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.

11.3.7 Place the column in a new 1.5 mL vial. Add 10-20 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.

Note: *For PCR positive control (methylation) and negative control (unmethylation), the primers for highly methylated sequences of H19ICR, LAP or XIST and the primer 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 the better PCR results.*

12. TROUBLESHOOTING

Problem	Cause	Solution
Little or No PCR Products	Insufficient cells.	Increase tissue amount (e.g. >1 million cells/per reaction).
	Insufficient cell lysis.	Follow the guidelines in the protocol. Check the cell lysis by observing a 5 µL portion of the tissue lysate under the microscope.
	Insufficient or too much sonication.	Follow the protocol instructions 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.
	DNA is not completely passed through the filter.	Increase centrifuge time to 1 minute at steps 9.5.3 to 9.5.7 of “Methylated DNA Isolation/Purification.”

Problem	Cause	Solution
Little or No Amplification Difference Between the Sample and the Negative Control.	Insufficient wash at each wash step.	Check if washing recommendations at each step is performed according to the protocol.
Little or No Amplification Difference Between the Sample and the Negative Control.	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.

13. NOTES

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