

# **ab117136 – Methylated DNA Immunoprecipitation (MeDIP) Kit - Tissue**

## 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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## 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 is an important pathway for the repression of gene transcription in diseased cells. 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.

ab117136 uses a proprietary and unique procedure/composition to enrich methylated DNA from various mammalian tissues. 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.

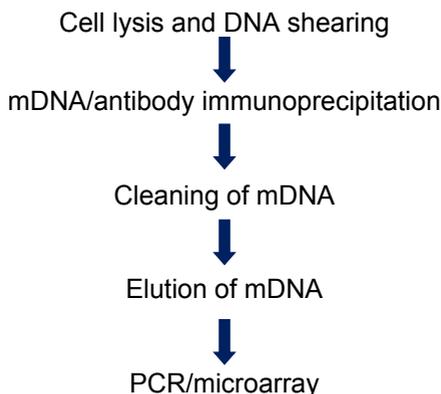
This kit has the following features:

- Directly immunoprecipitate methylated fractions of DNA from tissue lysates
- Highly efficient enrichment of methylated DNA: > 95%
- The fastest procedure available, which can be finished within 3.5 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

The Methylated DNA Immunoprecipitation (MeDIP) Kit - Tissue - contains all reagents required for carrying out a successful methylated DNA immunoprecipitation directly from mammalian tissues. 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    | 10 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 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 Lysis**

- 10.2.1 Place the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample. Weigh the sample and cut it into small pieces (1-2 mm<sup>3</sup>) with a scalpel or scissors.
- 10.2.2 Transfer tissue pieces to a Dounce homogenizer. Add 1 mL of the Homogenizing Buffer per every 200 mg of tissue and disaggregate tissue pieces by 10-30 strokes.
- 10.2.3 Transfer homogenized mixture to a 15 mL conical tube and centrifuge at 3000 rpm for 5 minutes at 4°C. If total mixture volume is less than 2 mL, transfer mixture to a 2 mL vial and centrifuge at 5000 rpm for 5 minutes at 4°C. Remove supernatant.

- 10.2.4 Add Lysis Buffer to re-suspend the disaggregated tissue pellet (100  $\mu$ L/20 mg tissues). Transfer the solution to a 1.5 mL vial (500  $\mu$ L maximum for each vial) and incubate at room temperature for 10 minutes and vortex occasionally.

## **11. ASSAY PROCEDURE**

### **11.1 DNA Shearing**

- 11.1.1 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  $\mu$ L of the sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp).
- 11.1.2 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  $-80^{\circ}\text{C}$  at this step). Dilute required volume of supernatant with ChIP dilution buffer at a 1:1 ratio (ex: add 100  $\mu$ L of ChIP dilution buffer to 100  $\mu$ L of supernatant).
- 11.2.2 Remove 5  $\mu$ L 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  $\mu$ L of Antibody Buffer by pipetting in and out.
- 11.2.4 Transfer 100  $\mu$ L of the diluted supernatant to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature ( $22-25^{\circ}\text{C}$ ) for 60 to 90 minutes on a rocking platform (50-100 rpm).
- 11.2.5 Remove supernatant. Wash the wells six times with 150  $\mu$ L of Wash Buffer. Allow 2 minutes on an orbital shaker (50-100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150  $\mu$ L of 1X TE Buffer.

## 11.3 Methylated DNA Isolation/Purification

- 11.3.1 Add 1  $\mu\text{L}$  of Proteinase K to each 40  $\mu\text{L}$  of DNA release buffer and mix. Add 40  $\mu\text{L}$  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  $\mu\text{L}$  of Reverse buffer to the samples; mix, and recover the wells with strip caps and incubate at 65°C in a water bath for 30 minutes. Also add 40  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\beta$ -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

| <b>Problem</b>   | <b>Cause</b>  | <b>Solution</b>  |
|--|---|--|
| Little or No PCR Product   | Insufficient tissues  | Increase tissue amount (ex: >20 mg tissues/per reaction)   |
|  | Insufficient tissue lysis   | Follow the guidelines in the protocol. Check the tissue lysis by observing a 5 $\mu$ L portion of the tissue lysate under the microscope |
|  | 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   |
| Little or No PCR Product   | 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 11.3.3 to 7 of "Methylated DNA Isolation/Purification"                                     |
| Little or No Amplification<br>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   |

## RESOURCES

| <b>Problem</b>   | <b>Cause</b>        | <b>Solution</b>  |
|--|---------------------|--|
| Little or No Amplification<br>Difference Between the Sample and the Negative Control | 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

# RESOURCES

# RESOURCES

# RESOURCES

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