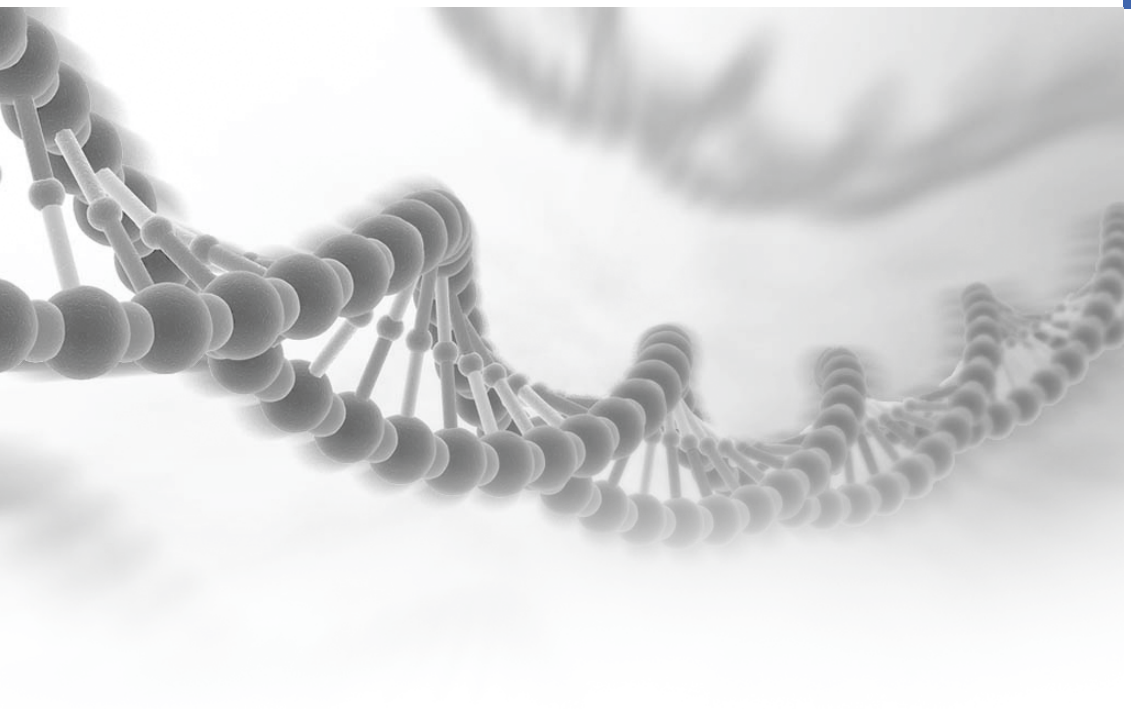
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleo-pore® DNA Methylation Kit



☐	**Nucleo-pore® DNA Methylation Kit**	**NP-6006D**	**50 Preps**

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Nucleo-pore®

Contents

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COMPONENTS

Kit contents

NUCLEOPORE® DNA METHYLATION KIT

Cat no	NP6006D (50 Prep)
Conversion Reagent CR *	5 Tubes
Dilution Buffer DL	1.5 ml
Dissolving Buffer DS	500 µl
Binding Buffer BB	30 ml
Wash Buffer WB (Conc) *	6 ml
Desulphonation Buffer DN	10 ml
Elution Buffer EB	1 ml
Spin Columns	50 pc.
Collection Tubes	50 pc

*Please see "Prearation and Storage of reagents"

Reagents, consumables and equipments not provided with the kit

- Ethanol (96-100%)
- 1.5ml microcentrifuge tubes
- Pipettes, vortex , Dry /WaterBaths

SAFETY INSTRUCTIONS

Always wear chemical resistant gloves, and safety goggles/face-mask/face shield when working with chemicals. Do not inhale or breathe vapor. Do not get into contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Keep reagents away from heat and open flame. Store in a cool dry place. Wash your hands thoroughly after handling reagents.

Conversion Reagent CR
Contain Sodium Metabisulfite. R 22, R31, R41, S26, S39, S46

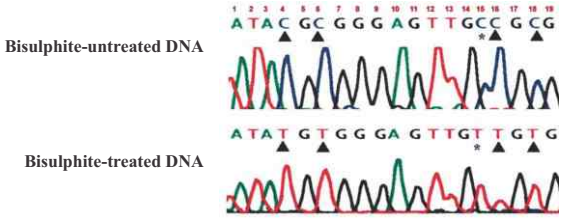
R 22 Harmful if swallowed, R 31 Contact with acids liberates toxic gas, R 41 Risk of serious damage to eyes; S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, S 39 Wear eye/face protection, S 46 If swallowed, seek medical advice immediately and show this container or label.

INTRODUCTION

Introduction to DNA Methylation

DNA methylation is a crucial part of normal organismal development and cellular differentiation in higher organisms. DNA methylation stably alters the gene expression pattern in cells It is an epigenetic event occurring naturally in both prokaryotic and eukaryotic system. In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA. The methylation of native DNA acts as a sort of primitive immune system, allowing the bacteria to protect themselves from infection by bacteriophage. and in higher eukaryotes DNA methylation functions in the regulation/control of gene expression. It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis Aberrant DNA methylation patterns have been associated with a large number of human malignancies and found in two distinct forms: hypermethylation and hypomethylation compared to normal tissue. Hypermethylation is one of the major epigenetic modifications that repress transcription via promoter region of tumour suppressor genes. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In many plants and animals, DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme . The majority of DNA methylation in mammals occurs in 5′-CpG-3′ dinucleotides, but other methylation patterns do exist. In fact, about 80 percent of all 5′-CpG-3′ dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes.

To date, a number of methods have been developed to detect/quantify DNA methylation of which the most common technique used today remains the bisulfite conversion method. This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil. Methylated cytosines remain unchanged during the treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing (see below) or other analytical procedures.



DNA sequencing results following bisulfite treatment. The methylated cytosine at position #15 remained intact while the unmethylated cytosines at positions #4, 6, 16 and 18 were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

Amplification of Bisulphite-Treated DNA

1. **Primer Design:** Generally, primers of 24 to 30 bases are required for amplification of bisulfite converted DNA. All non-methylated cytosine residues are converted into uracil during the bisulfite treatment in eukaryotes therefore, for primer design purposes all Cs should be treated as Ts. If the primer contains CpG dinucleotides with uncertain methylation status, then stringency can be reduced by mixed bases with C and T can be used. Usually, there should be no more than wobble per primer and they should not be be located toward the 3′ end of the primer.Optimal amplicon size should be between 150 - 300 bp; however larger amplicons (up to 1 kb) can be generated with proper optimization of the bisulfite reaction and PCR conditions. As most non-methylated cytosine residues are converted into uracil, the bisulfite-treated DNA usually is AT-rich and has low GC composition. Thus, it may be necessary to reduce the annealing temperature accordingly. Non-specific PCR amplification is relatively common with bisulfite treated DNA due to its AT-rich nature. PCR using “hot start” polymerases is strongly recommended for the amplification of bisulfite-treated DNA.

2. **Quantification :** Following bisulfite treatment of genomic DNA, non-methylated cytosine residues are converted into uracil. The recovered DNA is typically A, U, and T-rich. The original base-pairing no longer exists. Instead, it is single stranded with limited non-specific base-pairing at room temperature. The absorption coefficient at 260 nm resembles that of RNA. Use a value of 40 μg/ml for Ab260 = 1.0 when determining the concentration of the recovered bisulfite-treated DNA. The bisulfite-treated DNA can be visualized on agarose gels stained with ehidium bromide using trans-illuminator. It is recommended to cool the gel on ice (after electrophoresis) for 10-15 minutes prior to visualization will greatly enhance the resolution of the DNA.

Principle and Procedure

The Nucleo-pore® DNA Methylation Kit combines DNA denaturation and bisulfite conversion processes into one-step. This is accomplished by using temperature denaturation Methylation of DNA occurs on cytosine residues by the enzyme DNA methylases, especially on CpG dinucleotides. Such regions, with a GC content greater than 55% known as CpG islands are usually clustered near the regulatory region of genes. In addition to CpG, methylated cytosine residues are also found at CpNpG or CpNpN sites (N = A,T, or C) in plants.

Treatment of denatured DNA (i.e., single-stranded DNA) with sodium bisulfite leads to deamination of unmethylated cytosine residues to uracil, leaving 5-mC intact. The uracils are amplified as thymines, and 5-mC residues are amplified as cytosines in PCR. Method involves incubation of the target DNA with sodium bisulfite resulting in conversion of unmethylated cytosine residues into uracil, this leaves the methylated cytosines unchanged. Therefore, bisulfite treatment gives rise to different DNA sequences for methylated and unmethylated DNA. The Nucleo-pore® DNA Methylation Kit utilizes on-column desulphonation, eliminating tedious DNA precipitation steps. The kit is designed to minimize product degradation or loss during treatment and clean-up, to with complete conversion of unmethylated cytosines. . The eluted DNA is suited for all techniques currently used for the analysis of DNA methylation, including PCR, real-time PCR, MSP-PCR, bisulfate sequencing and Pyrosequencing and microarrays, etc.

Specifications

DNA Input: Samples containing 500pg - 2µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500ng.

Conversion Efficiency: >99% of non-methylated C residues are converted to U; >99% protection of methylated cytosines.

DNA Recovery: > 75%

Preparation and Storage of Reagents:

Conversion Reagent CR

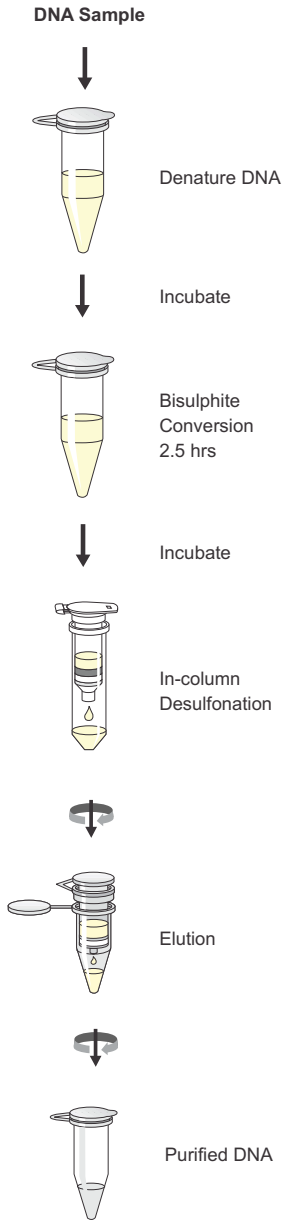
Add 900µl water, 300µl of Dilution Buffer DL and 50µl Dissolving Buffer DS to a tube of Conversion Reagent CR. Mix the solution at room temperature with pulse vortexing for upto 10 minutes.

Note:Small amount of undissolved reagent in the Conversion Reagent CR may be visible.Each tube of Conversion Reagent *CR can be used for 10 separate DNA treatments.* **Conversion Reagent solution** can be stored overnight at room temperature (in dark), one week at 4°C, or up to one month at -20°C.Pre-warm conversion reagent solution to 37°C.

Wash Buffer WB

Add 24 ml of 100% ethanol to the 6 ml **Wash Buffer WB** concentrate prior to use.

Bisulphite conversion of GC-rich DNA



PROTOCOL FOR DNA METHYLATION

Things to do before starting

- Check whether Conversion Reagent CR was prepared as per instruction
- Check whether Wash Buffer WB was prepared as per instruction

Procedure:

1. **Add 130 µl of the Conversion Reagent CR to 20 µl of DNA sample in a PCR tube.** If the volume of the DNA sample is less than 20 µl, make up the difference with water. Mix the sample by flicking the tube or pipetting the sample up and down, centrifuge to spin down the liquid to the bottom of the tube.

2. **Place the sample tube in a thermal cycler and perform the following steps:**
   98°C for 10 minutes
   64°C for 2.5 hours
   4°C hold (pause)

3. **Add 600 µl of Binding Buffer BB to a Spin Column placed into a Collection Tube.**

4. **Load the sample (from Step 2) into the Spin Column containing the Binding Buffer BB.** Close the cap and mix by inverting the column several times.

5. **Centrifuge at full speed (>10,000 x g) for 30 seconds. Discard the flow-through.**

6. **Add 100 µl of Wash Buffer WB to the column. Centrifuge at full speed for 30 seconds.**

7. **Add 200 µl of Desulphonation Buffer DN to the column and allow it to stand for 15 - 20 minutes at room temperature. After the incubation, centrifuge at full speed for 30 seconds.**

8. **Add 200 µl of Wash Buffer WB to the column. Centrifuge at full speed for 30 seconds.**

9. **Add another 200 µl of Wash Buffer WB and centrifuge for an additional 30 seconds.**

10. **Place the column into a 1.5 ml microcentrifuge tube. Add 10 µl of Elution Buffer EB directly to the column matrix. Centrifuge for 30 seconds at full speed to elute the DNA.**
    The DNA is ready for immediate analysis or can be stored at or below -20°C for later use. For long term storage, store at or below -70°C. The elution volume can be > 10 **µ**l depending on the requirements of your experiments, but small elution volumes will yield more concentrated DNA.

    Note: For DNA volumes >20 **µ**l, an adjustment needs to be made during the preparation of the **Conversion Reagent CR.** The amount of water is decreased 100 **µ**l for each 10 **µ**l increase in DNA sample volume. For example, for a 40 **µ**l DNA sample, 700 **µ**l of water is added to make the Conversion Reagent CR. The maximum DNA sample volume to be used for each conversion reaction is 50 **µ**l. Do not adjust the volumes of either the **Dissolving Buffer DS** or **Dilution Buffer DL.**
    Alternatively, water or TE (pH _ 6.0) can be used for elution if required for the experiment.

TROUBLESHOOTING GUIDE

Little or no recovery

Possible cause
- Conversion reagent not prepared correctly

Suggestions
- Check that Conversion reagent was prepared as per instructions

Possible Cause
- Wash Buffer WB not reconstituted

Suggestions
- Check that ethanol was added to Wash Buffer as per instructions

Poor Detection by PCR

Possible cause
- DNA is degraded, DNA quantity too low

Suggestions
- Prevent DNA degradation by maintaining a nuclease-free environment. Check DNA concentration by picogene instrument, or other spectrophotometer. Run target DNA on agarose gel to determine quantity, quality and size. If the DNA is from FFPE , it is already degraded. Check if sample was initially collected in buffered formalin.

Controls did not work, did not see bands as expected on gel

Possible cause
- DNA is degraded.

Suggestions
- Prevent DNA degradation by maintaining a nuclease-free environment.

Possible Cause
- PCR did not work

Suggestions
- Verify that all of the components have been added to the PCR reaction mix. Lower the annealing temperature of the reaction to 55 °C & standardize

Gene of interest not amplified, but control did

Possible cause
- DNA target does not contain sufficient amounts of CpG methylation.

Suggestions
- Raise input DNA concentration to at least 1 μg.

Possible Cause
- PCR did not work

Suggestions
- Verify that all of the components have been added to the PCR reaction mix. Optimize PCR conditions for target gene of interest

NO results in downstream PCR

Possible cause
- Little or no PCR product even in control reaction

Suggestions
- Confirm that the initial enzyme activation step was performed with hot-start PCR. Verify that all PCR components were added and that suitable cycling conditions were used.

Possible Cause
- Failure of convertion reaction

Suggestions
- Ensure that only high-quality DNA is used for the conversion reaction. See ordering information,for suitable Nucleopore kits for DNA purification.Ensure that all steps of the modification and cleanup protocol were followed.

Possible Cause
- Sample DNA was degraded before modification reaction.

Suggestions
- Ensure that sample DNA is handled and stored correctly.

Possible Cause
- PCR primers were not appropriate or incorrectly designed.

Suggestions
- Check primer design. Amount of template DNA used in PCR was insufficient. Increase amount of template DNA.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
® DNASure Tissue Mini Kit	50 preps	NP-61305
® DNASure Plant Mini Kit	50 preps	NP-79105
® DNASure Plant Mini Kit	250 preps	NP-79107
® DNASure Plant Midi Kit	20 preps	NP-78153
® DNASure Plant Maxi Kit	10 preps	NP-78164
® DNASure Blood Mini Kit	50 preps	NP-61105
® DNASure Blood Mini Kit	250 preps	NP-61107
® DNASure Blood Midi Kit	20 preps	NP-61184
® DNASure Blood Maxi Kit	10 preps	NP-61193
® DNASure Blood FastPure Kit	50 preps	NP-62205
® DNASure Blood FastPure Kit	250 preps	NP-62207
® SureSpin Plasmid Mini Kit	50 preps	NP-37105
® SureSpin Plasmid Mini Kit	250 preps	NP-37107
® SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
® SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
® SureSpin Buffer Set*	1	37107-BS
® SurePrep Plasmid Mini Kit	20 preps	NP-15123
® SurePrep Plasmid Mini Kit	100 preps	NP-15125
® SurePrep Plasmid Midi Kit	20 preps	NP-15143
® SurePrep Plasmid Midi Kit	100 preps	NP-15145
® SurePrep Plasmid Maxi Kit	10 preps	NP-15161
® SurePrep Plasmid Maxi Kit	25 preps	NP-15162
® SurePrep Plasmid Mega Kit	5 preps	NP-15183
® SurePrep Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleo-pore® DNA Methylation kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleo-pore® DNA Methylation kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleo-pore® DNA Methylation kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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