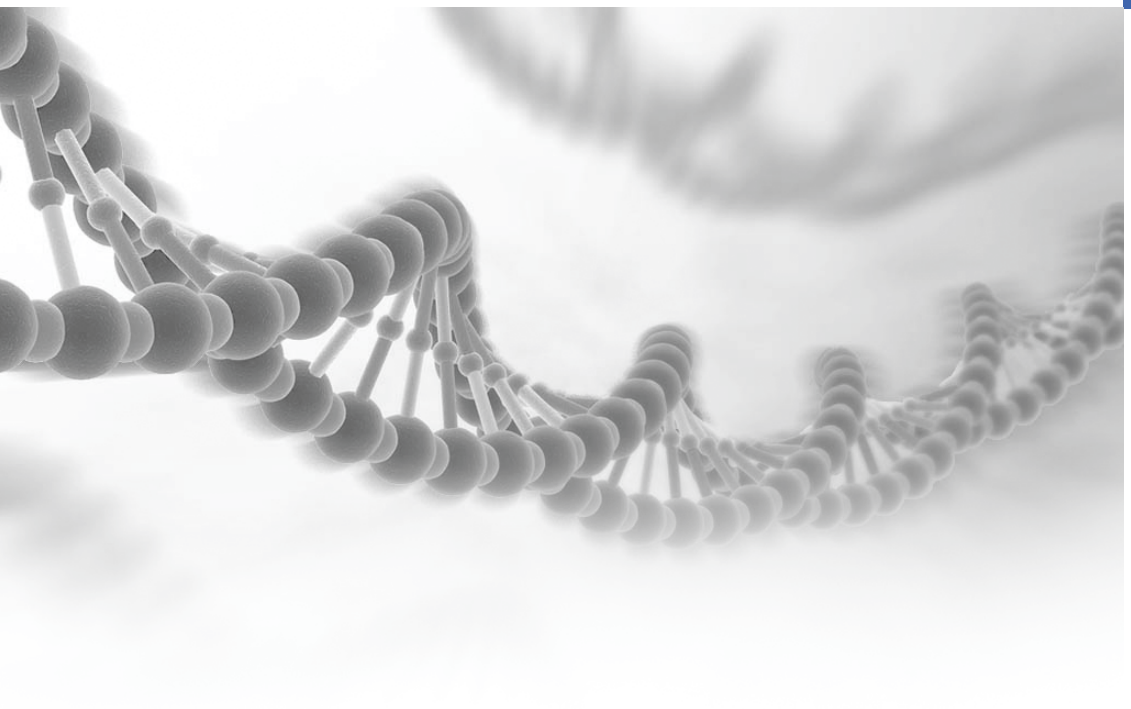
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleo-pore® Bisulphite DNA Clean-up Kit



☐ **Nucleo-pore® Bisulphite DNA Clean-up Kit** **NP-5205D** **50 Preps**

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Nucleo-pore®

Contents

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COMPONENTS

Kit contents

NUCLEOPORE® BISULPHITE DNA CLEAN-UP KIT

Cat #	NP-5205D (50 preps.)
Binding Buffer EBB	20 ml
Wash Buffer EBW (Concentrate)*	6 ml
Desulphonation Buffer EBD	10 ml
Elution Buffer EBE	1 ml
Fast Spin Column	50 columns
Collection Tubes	50 tubes
Instruction Manual	1

*Please see "Preparation and Storage of reagents"

Reagents, consumables and equipments not provided with the kit

- Absolute ethanol
- 1.5 ml microcentrifuge tube
- Tips
- Pipettes
- Centrifuge
- Vortex Mixer, Heating Block

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.

Binding Buffer EBB
Contain Guanidine Hydrochloride: R& S Phrases: R22-36/38

Desulphonation Buffer EBD
Contain Sodium Hydroxide: R & S Phrases: R35; S26, 37/39, 45

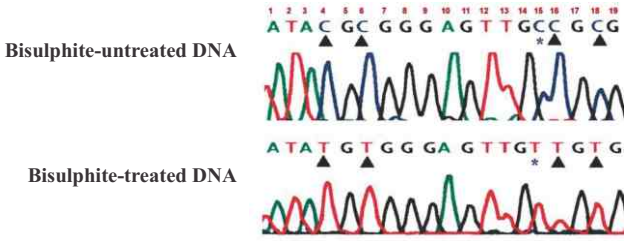
R22: Harmful if swallowed, R36/38: Irritating to eyes and skin, May cause sensitization bu inhalation and skin contact. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39 Wear suitable gloves and eye/face protection, S 45 In case of accident or if you feel unwell, seek medical advice immediately.

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 Bisulphite DNA Clean-up Kit has been specifically optimized for the purification of bisulfite-treated DNA from any in-house method or commercial reaction mixture containing bisulfite. The product features Fast-Spin, on-column desulphonation that minimizes DNA loss. The procedure fast and user friendly. Bisulphite-treated DNA purified with the Nucleo-pore Bisulphite DNA Clean-up Kit is ideal for PCR amplification for downstream DNA methylation analysis including endonuclease digestion, sequencing, microarrays, etc.

Specifications of Nucleo-pore Bisulphite DNA Cleanup Kit

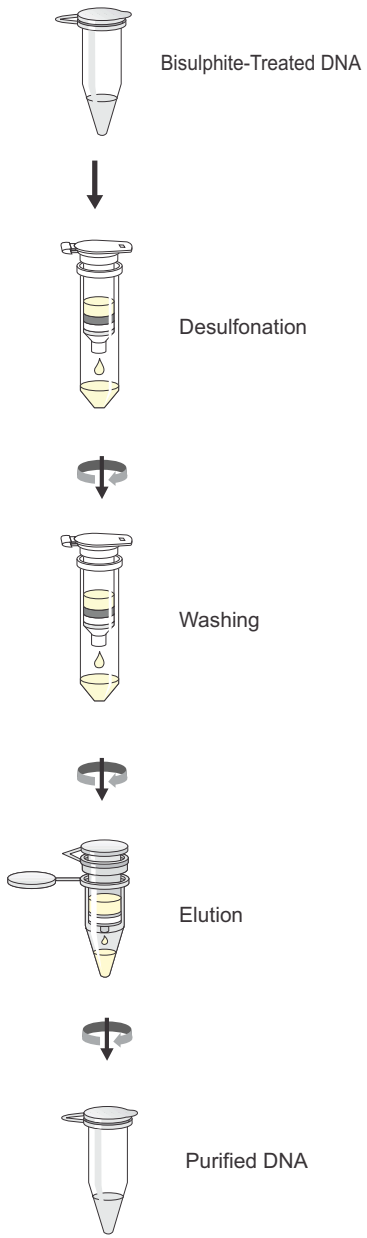
Starting amount: Up to 5 μg DNA can be taken from reaction mixtures containing bisulfite. Typical yield vary between 80and 90% in elution volume of $\geqslant$10 μl, Purified DNA can be used directly for PCR, endonuclease digestion, arrays, sequencing, etc.

Storage and Preparation of Reagents

Wash Buffer EBW

* Add 24 ml of absolute ethanol to the 6 ml Wash Buffer EBW before use. The Buffer is stable for upto one year at room temperature.

Bisulphite DNA Clean-up



PROTOCOL FOR CLEAN-UP OF BISULPHITE TREATED DNA

Things to do before starting

- Check whether wash buffer EBW was prepared as per instruction

Procedure:

1. **Add 4 volumes of Buffer EBB to each volume of a bisulfite-containing reaction mixture (4:1) and mix by tapping.**
2. **Apply the mixture onto a FastSpin Column in a Collection Tube. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through.**
3. **To the column add 100 µl of Wash Buffer EBW and centrifuge at 10,000 x g for 30 seconds.**
4. **Add 200 µl of Desulphonation Buffer EBD to the column, allow it to stand at room temperature for 15 - 20 minutes followed by centrifugation at 10,000 x g for 30 seconds.**
5. **Add 200 µl of Wash Buffer EBW to the column. Centrifuge at 10,000 x g for 30 seconds.**
6. **Repeat Step 5 with additional 200 µl of Wash Buffer EBW and centrifuge for 30 seconds.**
7. **Place the column in a fresh 1.5 ml microcentrifuge tube. Add 10 µl of Elution Buffer EBW directly to the column matrix. Centrifuge for 30 seconds at 10,000 x g 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.

TROUBLESHOOTING GUIDE

Poor Result in downstream application

Possible cause
- DNA purification not proper

Suggestion
- Make sure the Wash Buffer was re constituted as per instructions

Possible cause
- Carry over of chaotropic salts

Suggestions
- Check if centrifuge is provding enough gx force for proper centrifugation. Make sure second wash step(6) has been given PCR primers in correctly designed.

Poor DNA quality

Possible cause
- Ratio of Buffer EBB and reaction mixture not correct

Suggestion
- Make sure the ratio of Buffer EBB: Reaction Mixture is always 4:1

Too much of load on the column
- The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the column contents by the flowthrough.

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® Bisulphite DNA Clean Up 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® Bisulphite DNA Clean Up 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® Bisulphite DNA Clean Up 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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