

Instruction Manual Version 1 05.10



MagBisulfite kit

Magnetic bisulfite conversion kit

Cat. No. C02030010 (AF-106-0024)



Bring the power of magnetic separation
to bisulfite conversion



Content

Introduction	4
Kit Method Overview and time table	5
Kit Content	6
Required materials not provided	6
Protocol	7
Requirement for the starting material	7
Buffer preparation	7
STEP 1: DNA denaturation	8
STEP 2: DNA conversion	8
STEP 3: DNA purification and desulphonation	8
STEP 4: Control PCR	8
Troubleshooting Guide	10
Results	11
Ordering Information	13
Related Products	14

Introduction

DNA methylation plays a critical role in the regulation of gene expression. It is known to be an essential mechanism for guiding normal cellular development and maintaining of tissue identities. DNA methylation is the first discovered epigenetic mark, and remains the most studied. In animals, it predominantly involves the addition of a methyl group to the carbon-5 position of cytosine residues of the dinucleotide CpG. Many techniques have been developed to analyze DNA methylation. These methods can be divided into three groups: (1) chemical modification with bisulfite, (2) affinity-based isolation of methylated DNA and (3) treatment with methylation-sensitive restriction enzymes. Of these, bisulfite modification of DNA is a method most frequently used. Treatment of DNA with bisulfite converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected (Figure 1). Thus, bisulfite treatment introduces specific changes in the DNA sequence that depends on the methylation status of individual cytosine residues, yielding single nucleotide resolution information about the methylation status. Various analyses can be performed on the altered sequence to retrieve this information: bisulfite sequencing, methylation-specific PCR, high resolution melting curve analysis, microarray-based approaches, next-generation sequencing.

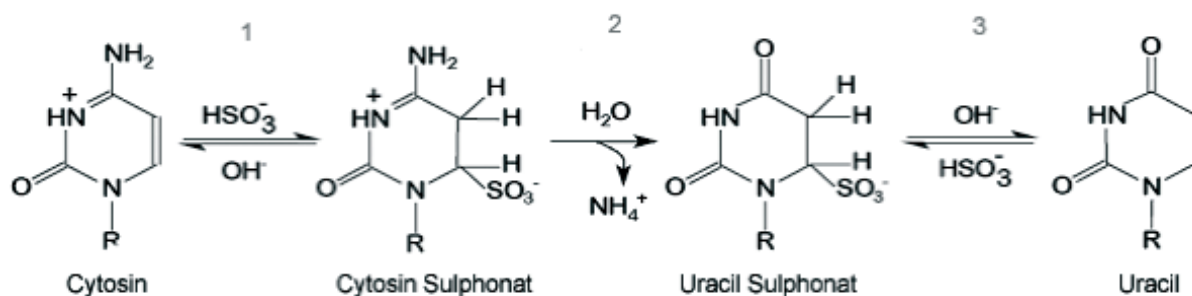


Figure 1. Bisulfite-mediated conversion of cytosine. Bisulfite conversion is performed under acidic conditions and preferentially deaminates cytosine in a nucleophilic attack whilst the methyl group on 5-methylcytosine is protecting the amino group from the deamination. Step 1: Sulphonation; Step 2: deamination; Step 3: desulphonation.

Kit Method Overview and time table

In this protocol, sodium bisulfite is used to convert cytosine residues to uracil residues in single-stranded DNA, while 5-methylcytidine remains non-reactive. Optimized reagents ensure the complete conversion and minimize DNA degradation during the treatment. The converted DNA is then purified with innovative IPure magnetic beads and desulphonated directly on the beads. The use of IPure magnetic beads results in the efficient DNA recovery. The isolated DNA is suited for down-stream applications used for analysis of bisulfite-converted DNA.

The protocol includes the following steps:

- I. DNA denaturation
- II. DNA conversion
- III. DNA purification and desulphonation
- IV. Control PCR (optional)

Bisulfite conversion kit protocol: steps and time table.

	Protocol step	Sample volume	Time needed
STEP 1-	DNA denaturation	25 µl of DNA	30 min
STEP 2-	DNA conversion	25 µl of denaturated DNA 200 µl of Conversion Reagent	1 h
STEP 3-	DNA purification and desulphonation	225 µl of Converted DNA 30 µl of Magnetic beads ↓ 3x washes desulfonation ↓ 3x washes DNA Elution ↓ 50 µl of purified DNA	2 h
STEP 4-	Control PCR	5 µl	3 h

Kit Content

Component	Quantity	Storage
Conversion Reagent 1	Mix for 12 reaction x 2	RT , protected from light
Conversion Reagent 2	2.5 ml x 2	RT , protected from light
IPure beads	800 µl	4°C, do not freeze
Carrier	50 µl	-20°C
Wash buffer	10 ml	RT
DE buffer	1.5 ml	RT, tightly close bottle
Elution buffer	1.5 ml	RT
Bisulfite-specific primer pair	30 µl	-20°C

The Bisulfite conversion kit is shipped at room temperature. Upon arrival, carrier and primer pair should be stored at -20°C, IPure beads at 4°C.

Required materials not provided

Equipment

- Nuclease-free barrier tips
- Nuclease-free 1.5 ml tubes
- Vortex mixer
- Thermo Mixer or equivalent (required temperature 42°C and 70°C)
- Bench top centrifuge for 1.5 ml-tubes
- Rotating wheel
- Magnetic rack for 1.5 ml tubes (optional)
- Agarose gel equipment

Reagents

- Nuclease-free water
- Isopropanol 100%
- PCR reagents
- Sodium hydroxide, Molecular Biology grade

Protocol

Requirement for the starting material:

- Pure DNA should be used (A260/280 ratio between 1.7 and 1.9).
- DNA should be diluted in water or TE buffer
- Optimal quantity of DNA is 100 ng - 1 µg
- Lowest limit of DNA quantity is 1 ng
- Maximum volume of DNA sample is 22.5 µl

Buffer preparation before starting the protocol:

- Prepare fresh solution of 3M sodium hydroxide (not provided).
- Prepare the Complete Conversion Reagent by adding Conversion reagent 2 (liquid) to Conversion reagent 1 (powder). Mix briefly by vortexing and heat in the boiling water for 3-5 min till complete salt dissolving. Keep at 70°C until use to avoid any salt precipitation. One tube of Complete Conversion reagent is enough for 12 reactions. Protect the solution from light. Use only fresh solution!
- Prepare Complete Wash Buffer by adding 10 ml of isopropanol (100%) to the Wash Buffer before use. Make a label on the bottle to indicate that isopropanol has been added. Store the Complete Wash Buffer at RT in a tightly closed bottle.
- Prepare Complete DE buffer by adding 1.5 ml of isopropanol (100%) to the DE buffer before use. Make a label on the bottle to indicate that isopropanol has been added. Store the Complete DE buffer at RT in a tightly closed bottle.

STEP 1. DNA denaturation



Note: DNA has to be in single-stranded conformation to ensure the complete conversion.

1. Place 1 ng - 1 µg of DNA in a nuclease-free 1.5 ml tube and add nuclease-free water to DNA sample to bring the volume to 22.5 µl.
2. Add 2.5 µl of freshly prepared 3M sodium hydroxide. Mix samples by vortexing and spin down.
3. Incubate at 42°C in ThermoMixer for 30 min.
4. Proceed directly to the next step (DNA conversion).

STEP 2. DNA conversion



5. Use freshly prepared Complete Conversion Reagent as described in the Buffer Preparation section.
6. Add 200 µl of Complete Conversion Reagent to the tubes containing the denatured DNA.
7. Mix and incubate samples at 70°C for 1 hour. Protect from light.
8. Proceed directly to the DNA purification and desulphonation. Converted DNA should be processed as soon as possible.

STEP 3. DNA purification and desulphonation



Note: It is important to ensure the complete removal of sulphate group (desulphonation), otherwise it can inhibit the down-stream applications.

9. Spin briefly the sample and add the following components to each of the reaction tubes containing converted DNA:
 - 2 µl of carrier
 - 30 µl of resuspended IPure beads (vortex each time before pipetting)
10. Incubate for 30 min at RT with gentle rotation.
11. Precipitate the IPure beads either in a Magnetic Rack or by centrifugation as described above and discard the liquid by pipetting.
12. Add 100 µl of Complete Wash buffer, mix by pipetting and rotate for 3 min at RT.

An appropriate 1.5 ml-tube magnetic rack can be used to precipitate Magnetic beads. Spin briefly the tubes by centrifugation (10 sec) at 5000 rpm and put them in the rack. Wait for 1 min and discard the liquid by pipetting. Otherwise, IPure Magnetic Beads can be precipitated by centrifugation for 5 min at 5000 rpm at RT.

13. Precipitate the IPure Magnetic beads and discard the liquid.
14. Repeat steps 12-13 two times.
15. Add 100 µl of Complete DE buffer to the IPure Magnetic beads and resuspend the beads by vortexing.
16. Incubate for 30 min at 42°C with shaking in Thermo Mixer at 1300 rpm.
17. Precipitate IPure beads and wash them three times with Complete Wash Buffer as described in steps 12-13.
18. Carefully remove all traces of Complete Wash Buffer (10 µl pipette should be used) and dry the beads for 3 min at 42°C with open caps.
19. Add 50 µl of Elution buffer, mix well and rotate at RT for 30 min.

Note: if down-stream application is sensitive to EDTA presence, DNA might be eluted with nuclease-free 10 mM tris pH 8.0 (not supplied).

20. Precipitate the IPure beads and transfer the liquid containing converted DNA into new nuclease-free tube.
21. Store DNA at 20°C/-80°C. Avoid multiple freeze-thaw cycles.

STEP 4. Control PCR



(optional – use control bisulfite-specific primer pair to confirm conversion)

Step IV consists of Control PCR with bisulfite-specific primer pair designed for the amplification of human bisulfite-converted DNA. Primers span CpG-rich region but do not contain any CpG in their sequences. The primers have been designed to amplify a PCR product of 204 bp, (genomic locus chr16: 3008254-3008458).

Note: The choice of DNA polymerase for PCR amplification of bisulfite-converted DNA is essential. MethylTaq DNA polymerase (Cat. No. AF-103-0250) is recommended to avoid uracil stalling.

22. Use 2-5 µl of bisulfite converted DNA per PCR.
23. Use 1 µl of primers per reaction.
24. Perform PCR as follow:

1 cycle	at 95°C for 10 min
40 cycles:	95°C for 30 sec
	57°C for 1 min
	72°C for 1 min
1 cycle	at 72°C for 3 min
Hold at 20°C	

25. Analyse the amplified PCR product in 1.5 -1.8 % agarose gel.

Troubleshooting Guide

Problem/Question	Recommendation
Incomplete conversion	<ul style="list-style-type: none"> • Check the purity of DNA sample. Proteins and RNA contaminations affect the conversion rate. • DNA is not completely denaturated: make sure that freshly prepared 3M NaOH is used. • Ensure that no more than 1 µg of DNA is used per reaction. The excess of DNA in conversion reaction reduces the efficiency.
No PCR products in control reaction	<ul style="list-style-type: none"> • Make sure that DNA polymerase used is not inhibited by uracil in the converted sequence. Non-proofreading Hot Start Taq-polymerase is recommended. • Bisulfite conversion reagent prepared/stored incorrectly. Ensure that only freshly prepared Conversion Reagent is used. • Amount of starting DNA is outside of recommended range. • Incomplete desulphonation: be sure that desulfonation reaction is carried out at 42°C.
Amplification with control primers but not with your own primers	<ul style="list-style-type: none"> • Check the primers design and PCR conditions. • Some free on-line programmes are available for primers design: http://www.urogene.org/methprimer or http://bisearch.enzim.hu

Results

Highly efficient conversion

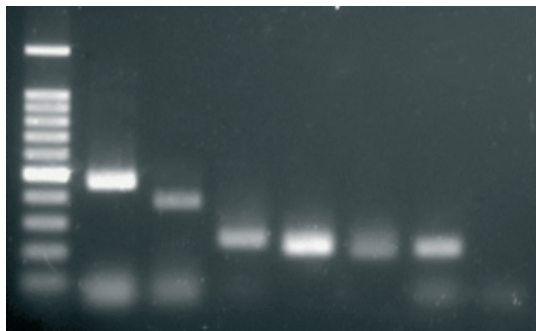


Figure 1. Different genomic loci are efficiently converted using the MagBisulfite kit. 1 µg of genomic DNA from MCF7 cell line was converted using the MagBisulfite kit. Six individual bisulfite-specific primer pairs were used for PCR amplification with Diagenode MethylTaq DNA polymerase (Cat. No. AF-103-0250). PCR products are visualized in 1.5% agarose gel stained with SYBR Safe.

Sensitivity of detection

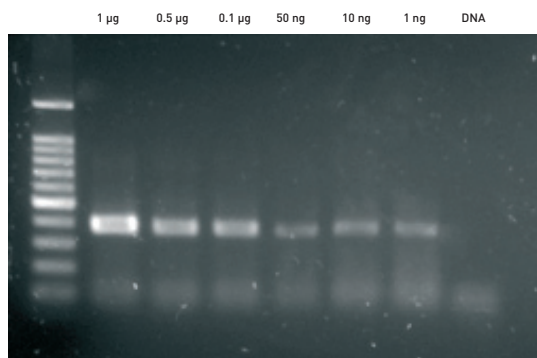


Figure 2. Genomic DNA from MCF7 cell line was extracted using Diagenode GenDNA extraction module (Cat. No. mc-magme-003). Various amounts of DNA as indicated were converted using the MagBisulfite kit. 5 µl from each sample were amplified by end-point PCR for 40 cycles with Diagenode MethylTaq DNA polymerase (Cat. No. AF-103-0250). PCR product (363 bp) is visualized in 1.5% agarose gel stained with SYBR Safe. **As little as 1 ng is sufficient for the conversion using Diagenode Bisulfite Conversion kit.**

Efficient DNA recovery with IPure beads

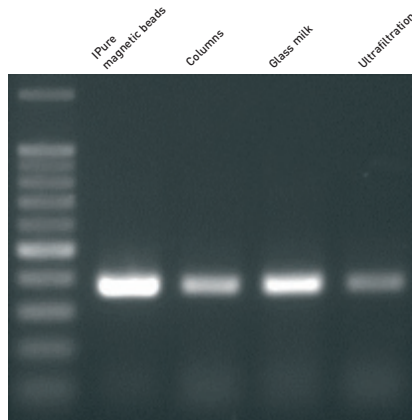


Figure 3. Human Genomic DNA was converted with Diagenode Bisulfite Conversion kit and purified with IPure Magnetic beads, columns-based purification, GlassMilk or ultrafiltration with Microcon centrifugal filters. IPure magnetic beads purification results in better DNA recovery after bisulfite conversion.

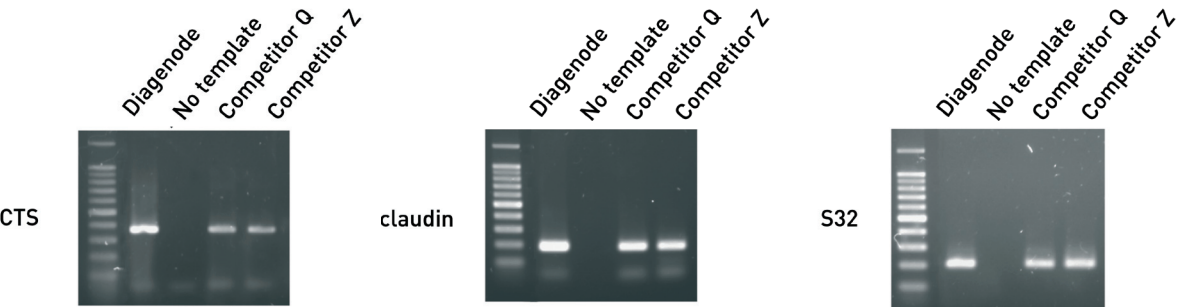


Figure 4. High yield of converted DNA with MagBisulfite kit comparing to competitors.

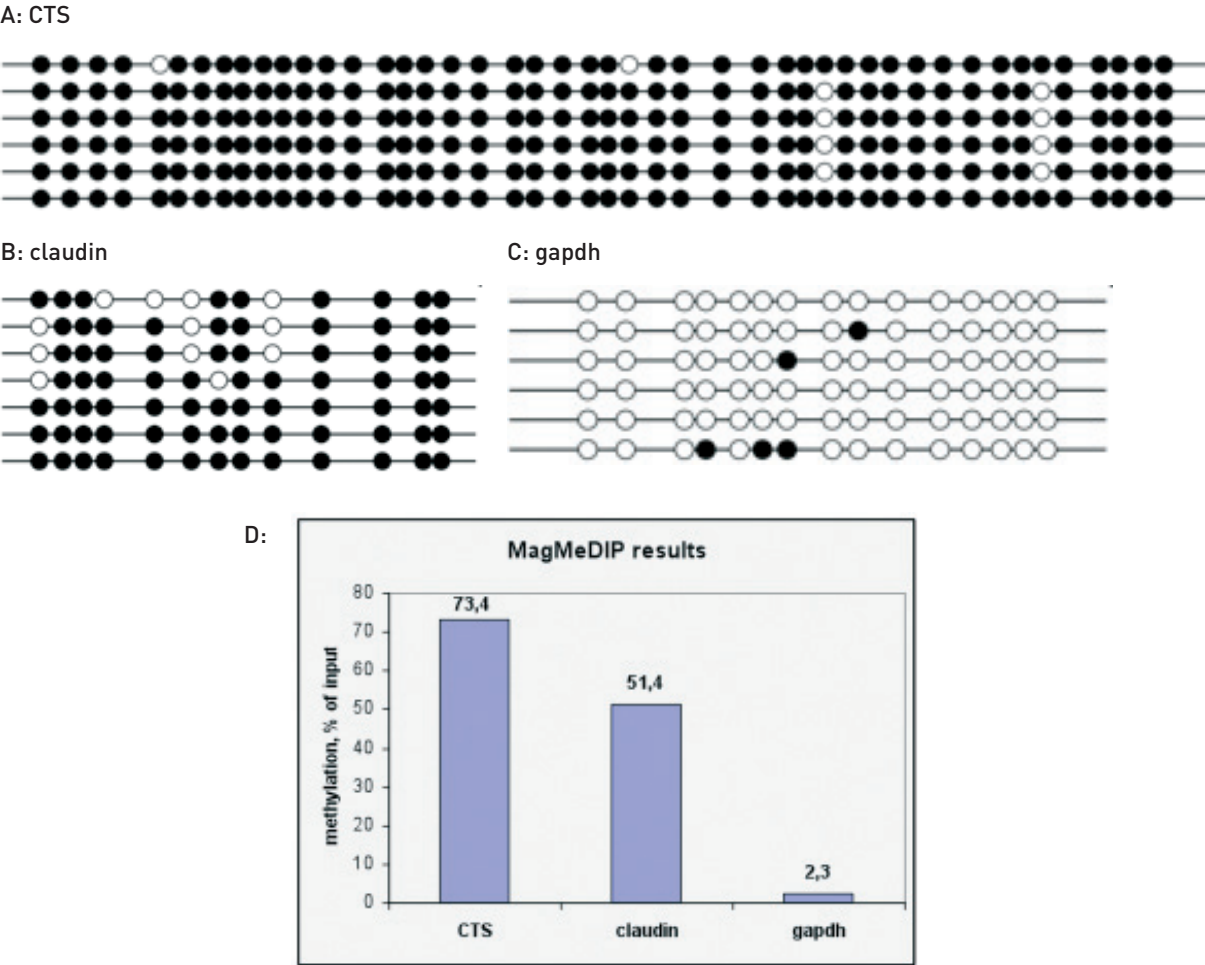


Figure 5. Methylation profiling by bisulfite sequencing comparing to MagMeDIP kit. 1 µg of human genomic DNA from MCF7 cells was converted with Diagenode Bisulfite conversion kit and sequenced to analyze the methylation status of 3 genomic loci. A: CTS region is highly methylated in MCF7 cells. B: claudin region is medium methylated region. C: gapdh is unmethylated region. D/ methylation profile is the same with MagMeDIP kit.

Ordering information

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BIORUPTOR® SONICATION

Ideal for DNA and Chromatin Shearing as well as for cell and tissue disruption. With hundreds of publications the Bioruptor® has acquired an unmatched reputation in the scientific community.

- Simple operating procedure
- Sonication in sealed tubes
- Better reproducibility of results
- Validated ChIP and MeDIP protocols

Note: database of shearing protocols available on our website.

Diagenode's Bioruptor® sonicators (Bioruptor®, Bioruptor® Next Gen, Bioruptor® Twin, Bioruptor® XL) use a unique system to uniformly process multiple samples simultaneously in sealed tubes of 0.5 ml to 50 ml capacity. It consists of a high power ultrasound generating element which is located below a water bath. The frequency of the ultrasound energy produced by the Bioruptor® is 20 kHz, which is similar to a probe sonicator.

The Bioruptor® allows you to sonicate 6 to 48 sealed tubes simultaneously without aerosol formation improving biosafety (e.g. Mycobacterium, Viruses, etc.). The continuous sample rotation guarantees equal distribution of the energy and therefore 100% reproducible results. The parameters can be efficiently controlled and allow for the automation of the sonication step in your experiments. The Bioruptor® is easy to setup and uses standard disposable containers (PCR tubes, Eppendorf, 15 ml and 50 ml Falcon/Corning tubes). Validated protocols (e.g. ChIP, MeDIP etc.) can be standardized and transferred between labs.

What are the effects of ultrasound on biological samples?

High powered ultrasound waves can produce gaseous cavitation in liquids. Cavitation is the formation of small bubbles of dissolved gases or vapors due to the alteration of pressure in liquids. These bubbles are capable of resonance vibration and produce vigorous eddying or microstreaming. This mechanical stress has multiple effects on biological samples including; effective cell lysis, DNA and chromatin shearing as well as homogenization.

When using a probe sonicator, the microstreaming phenomenon is limited to the vicinity of the probe which can generate high amounts of heat and release metal fragments. In contrast, the Bioruptor's water bath is equally exposed to ultrasound energy allowing for the dissipation of heat and providing uniform absorption of energy.



Major benefits of the Bioruptor®

- Reproducibility
- Time-saving
- Automated and High Throughput
- No "foaming"
- No risk of contamination between samples
- Wide range of sample size (10 µl - 20 ml)
- Validated for ChIP, MeDIP & Shotgun libraries (sequencing)



STANDARD	STANDARD	HIGH THROUGHPUT	HIGH THROUGHPUT
Bioruptor® (UCD-200) → Number of tubes to be processed simultaneously: 12 (0.5 ml); 6 (1.5 ml); 6 (15 ml); 3 (50 ml) → Analog timer → Plastic (or metal upgrade) soundproof box	Bioruptor® Next Gen (UCD-300) → Number of tubes to be processed simultaneously: 12 (0.5 ml); 6 (1.5 ml); 6 (15 ml); 3 (50 ml) → Digital timer → Metal soundproof box → Overheat shutdown protection → Possibility to control cooling system → Machine lifetime control	Bioruptor® Twin (UCD-400) → Number of tubes to be processed simultaneously: 24 (0.5 ml); 12 (1.5 ml); 12 (15 ml); 6 (50 ml) → Digital timer → Metal soundproof box → Overheat shutdown protection → Possibility to control cooling system → Machine lifetime control	Bioruptor® XL (XL-2006) → Number of tubes to be processed simultaneously: 48 (0.5 ml); 24 (1.5 ml); 24 (15 ml); 12 (50 ml) → Digital timer → Metal soundproof box Ideal for cell lysis. Not validated for all kind of chromatin samples.
Validated for ChIP, MeDIP, MBD & Shotgun libraries (sequencing)			
Compatible with the SX-8G IP-Star® Automated System for ChIP, MeDIP & MBD			

Next Generation Sequencing

“Although many options exist, we routinely generate high-quality libraries using a focused acoustics system (Covaris) or a closed system ultrasonic disruptor (**Bioruptor®, Diagenode**). The latter system is **lower in cost**, while achieving higher energy transfer efficiency and **more reproducible** performance than standard probe sonicators. In addition, multiple samples can be processed simultaneously in a uniform manner.”
(From Hodges et al. (*Nature Protocols* **4**, -960-974, 2009) describing preparation of DNA for high-throughput sequencing)

Here at Diagenode we are committed to remain the “**best-in-class**” epigenetic kits supplier by meeting the epigenetic research community needs.

Diagenode's kits are easy-to-use and will deliver rapid, sensitive and reproducible results. They are designed to support every step of your experiment, save you time and require minimal starting material. We have kits to perform individual steps of your experiment or cover the complete assay from start to finish. The two techniques that we are currently placing a large emphasis on are the ChIP and MeDIP assays.

CHROMATIN FUNCTION

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) is a method used to determine the location of DNA binding sites on the genome for a particular protein of interest. ChIP assay offers a huge potential to improve knowledge about the regulation of the genome expression. This technique is now used in a variety of life science disciplines and addresses several essential questions; for example cellular differentiation, tumor suppressor gene silencing as well as the effect of histone modifications on gene expression.

ChIP (crosslinked) procedure

Chromatin-bound proteins are formaldehyde fixed to the DNA. The chromatin is then sheared to small fragment sizes (200 bp – 1 kb) and immunoprecipitated using a specific ChIP-grade antibody. Following reverse crosslinking and proteinase K treatment, the purified DNA is analyzed to identify the genomic regions that the specific protein is bound to.

ChIP, ChIP-on-chip and ChIP-Seq are used to investigate interactions between proteins and DNA in vivo. It allows the identification of binding sites of DNA-binding proteins both efficiently and quantitatively. These protocols have been optimized to analyze proteins closely bound to the chromatin, including transcription factors, replication-related proteins, histones, histone variants and histone modifications.

ChIP combined with microarray hybridization (ChIP) or sequencing (Seq) can localize protein binding sites which may help in identifying functional elements in the genome (whole-genome or specific genomic regions).

All our products have been extensively validated in ChIP using various protein targets. The combination of our kits, reagents and equipment is the perfect starting point to your ChIP success.

	Magnetic bead-based ChIP		Agarose bead-based ChIP	
	Automated ChIP		Manual ChIP	
	Auto ChIP kit	HighCell# ChIP kit	LowCell# ChIP kit	Transcription ChIP kit
Features	Use from 1,000 - 5 million cells per IP, validated on SX-86 IP-Star® Automated System which ensures maximum reproducibility and time savings	Use from 1 - 10 million cells per IP, ideal to recover large amounts of DNA (transcription factors, ChIP-on-chip) and to avoid bias due to amplification steps	Use from 1,000 - 1 million cells per IP, fast, increased DNA yield.	Standard protocol (3 days) optimized for transcription factors.
Optimized for	All DNA-protein interactions	All DNA-protein interactions	All DNA-protein interactions	Histones & histone modifications
Suitable for ChIP-seq and ChIP-on-chip	yes (1)	yes (1)	yes (1)	yes
Amount of cells/IP	1,000 - 5 million	1 - 10 million	1,000 - 1 million	100,000
Time from cell collection to PCR	1 day	1 day	1 day	3 days
Buffers & Reagents for	IP, DNA purification	Cell lysis, chromatin shearing, IP, DNA purification	Cell lysis, chromatin shearing, IP, DNA purification	Cell lysis, chromatin shearing, IP, DNA purification
Control antibodies	anti-IgG (rabbit)	anti-IgG (rabbit)	anti-IgG (rabbit)	anti-H3K4me3
Control PCR primer pairs	-	human TSH2B, c-fos, myoglobin exon 2	human SAT2, c-fos, myoglobin exon 2	BMX / c-fos / beta actin / myoglobin exon 2
DNA purification	DNA isolation buffer	DNA isolation buffer	DNA isolation buffer	Not included
Format	16 or 100 rxns	16 rxns	16 or 48 rxns	18 rxns
				DNA purifying slurry
				60 or 180 rxns

(1) DNA purification has to be carried out with the IPure kit (Cat. No. AL-100-0100; not included in the kit)

(2) Please mention your choice of antibody on your purchase order.

DNA METHYLATION

DNA is methylated following DNA replication and is involved in a number of biological processes including; regulation of imprinted genes, X chromosome inactivation and tumor suppressor gene silencing in cancer cells. Methylation often occurs in cytosine-guanine rich regions of DNA (CpG islands), which are commonly upstream of promoter regions. Here at Diagenode we have developed a variety of assays and kits to facilitate the research of DNA methylation.

DNA methylation and CpG islands

DNA methylation is an epigenetic event that affects cell function by altering gene expression. It involves the addition of a methyl group to cytosine residues at CpG dinucleotides, a reaction that is catalyzed by DNA methyltransferase (DNMT) enzymes. The DNMT enzymes (DNMT 1, 3a or 3b) catalyses the transfer of a methyl group (CH₃) from S-adenosylmethionine (SAM) to the carbon-5 position of cytosine generating 5-methyl cytosine.

There are about 45,000 CpG islands in the human genome, and these are mostly located at promoters within first exons of genes. CpG islands are unmethylated in normal cells and their methylation has been associated with human disorders and epigenetic diseases. CpG islands located in the 5' region of a gene are known as molecular switches that can turn-off or turn-on the expression of the downstream gene. For the majority of genes, the CpG islands in their 5' regions are not methylated and they are ready to be expressed.

In some cancers, CpG islands in the 5' regions of tumor-suppressor genes are methylated and their expressions are switched-off. For example the methylation of CpGs near tumor suppressor genes (p53 or p16) has been linked to their transcriptional silencing and can result in cancerous tumors. This mechanism of gene silencing is known to be one of the major mechanisms of tumor-suppressor gene inactivation.

What is the role of DNA methylation?

In higher organisms from plants to humans, methylation protects DNA from endonuclease degradation and plays a critical role in regulating gene expression, making it essential for normal development and function.

DNA methylation, Epigenetics and cancer

The pattern of DNA methylation and histone modification(s) is critical for genome stability and controlling gene expression in the cell and plays an essential role in maintaining cellular function. The formation of cancer cells can be driven by the alteration of the DNA methylation pattern leading to the silencing of tumor suppressor genes. For example hyper-methylation at the promoter regions of BRCA1, hMLH1, p16INK4a and VHL lead to gene silencing and catastrophic consequences.

On the other hand, global hypo-methylation, which can lead to chromosome instability, has also been recognized as a cause of oncogenesis. This is because it is often accompanied by region-specific hyper-methylation and therefore disrupts the balance of the cells gene expression.

Preclinical and clinical studies suggest that part of the cancer-protective effects associated with several bioactive food components may relate to DNA methylation patterns (Davis and Uthus 2004).

Diagenode proposes different methods and kits for the study of DNA methylation patterns.

	Auto MethylCap kit*	MethylCap kit	Auto MeDIP kit*	MagMeDIP kit	MeDIP kit	MethylEasy Xceed kit	MethylEasy kit
Features	Allows for fractionation of methylated DNA by CpG density, validated on SX-8G IP-Star Automated system, includes control primer pairs for assessment of capture efficiency	Allows for fractionation of methylated DNA by CpG density, magnetic beads permit fast and sensitive capture, includes control primer pairs for assessment of capture efficiency	Quality control using internal controls, validated on SX-8G IP-Star Automated System, high specificity (monoclonal 5-mec Ab)	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-mec Ab), fast	Quality control using internal controls, high specificity (monoclonal 5-mec Ab), agarose, beads, fast	Gives precise information on methylation status of single cytosines	Gives precise information on methylation status of single cytosines
Suitable for	qPCR, linear amplification and genome-wide analysis (microarray, sequencing)	qPCR, linear amplification and genome-wide analysis (microarray, sequencing)	qPCR, linear amplification and genome-wide analysis (microarray, sequencing)	qPCR, linear amplification and genome-wide analysis (microarray, sequencing)	qPCR, linear amplification and genome-wide analysis (microarray, sequencing)	qPCR, sequencing, microarray	qPCR, sequencing, microarray
Amount of DNA/rnx	1µg	1µg	1µg	1µg	1µg	Min 50 pg	Min 100 pg
Total Time of Assay	1 day	1 day	1 day	3 days	3 days	90min (without primer design and sequencing)	6h – 18h (without primer design and sequencing)
Controls	GST-protein (not included)	GST-protein (not included)	methylated and unmethylated BAC clones	methylated and unmethylated BAC clones	methylated and unmethylated BAC clones	untreated DNA and bisulphite treated DNA	untreated DNA and bisulphite treated DNA
#rxns per kit	48	48	16 or 100	10 or 48	10	40	25 or 96

* Validated on SX-8G IP-Star Automated System.

Diagenode is recognized as the “**Best-in-class**” Epigenetic Antibody Company by EU programs, Academic labs and Biotech companies.

Research continues to demonstrate the importance of histone modifications and how they can regulate gene expression. These include their interplay with histone variants, DNA methylation, transcription factors, RNA components and ATP-dependent chromatin remodeling or assembly factors.

There are tremendous opportunities offered by the use of immuno-techniques like immunoprecipitation assays (especially in combination with DNA microarrays and High Throughput sequencing technologies). At Diagenode we know that a successful immunoprecipitation requires an effective, specific and high quality antibody. Therefore we focus our efforts on ensuring all of our antibodies meet our stringent quality control standards.

Diagenode is involved in several European programs; often requiring us to produce high quality antibodies for the epigenetic field. This has allowed us to develop our antibody production and characterization procedures together with academic researchers. Therefore guaranteeing our antibodies meet the standards required by you; the researcher.

Selection of our Antibodies

The production of our antibodies is selected through a number of sources including:

- Direct collaborations (including European programs)
- Customer suggestions (licensing)
- Research focus areas
- Conferences

The majority of our antibodies are developed and characterized in-house. These are complemented with high quality antibodies sourced from academic laboratories or primary manufacturers.

Antibody production, characterization and quality controls at Diagenode

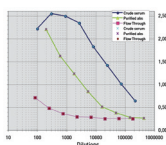
Below is an outline of our robust antibody production procedures, which have been developed in collaboration with our academic partners. Most of our antibodies are directed against epigenetic specific targets; including histone modifications, chromatin modifying and/or interacting proteins as well as transcription factors. Diagenode's goal is to characterize every batch of antibody using established QC procedures and therefore maximizing the reproducibility of our antibody between batches.

- 1. Peptide design** – Our R&D department has developed techniques to increase the immunogenicity of our peptides to increase the titre and specificity of our antibodies.
- 2. Titre analysis** – We analyse the immune-response in the crude sera by performing peptide-ELISA.
- 3. Affinity purification** – This is performed using the immunizing peptide.
- 4. Characterization** – The characterization of an antibody is the most important step of this process and we will commonly test using Peptide ELISA, Western Blot, Immunofluorescence and / or Dot Blot techniques.
- 5. ChIP-Grade validation** – Specific antibodies will be characterized using Chromatin Immunoprecipitation techniques with all our data available on our antibody datasheets.
- 6. Diagenode Next Generation Characterization** – Specific antibodies will be characterized using our in-house Illumina Illumina Instrument (ChIP-seq) and / or through collaborative agreements using microarray approaches (ChIP-on-chip).

QC flowchart (step by step)

CRUDE SERA

- Immune response
- Screening different bleeds, comparison



SINGLE AFFINITY PURIFIED

- QC purification
- Checking response in comparison with crude serum
- Fine titration

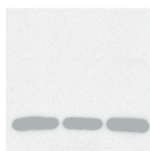
Dot Blot



- Specificity
- Screening targeted cross reactions

- Specificity
- Screening targeted cross reactions

Western Blot

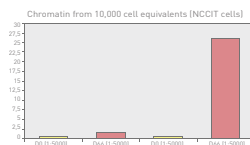


- Testing global specificity
- Immunofluorescence if applicable

- Testing global specificity
- Immunofluorescence & ELISA inhibition if applicable

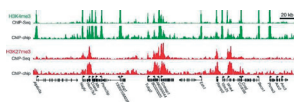
Chromatin IP

- Chromatin IP
- Screening of dilutions of different bleeds



- Chromatin IP
- Checking efficiency and quality of crude sera / purified Ab

ChIP-chip / ChIP-seq



Antibodies that have been consistent in ChIP are applied in the technique of ChIP-on-chip and ChIP-seq through collaborative agreement.

DIAGENODE HEADQUARTERS

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