Instruction Manual

Chromatin shearing optimization kit

Medium SDS

Cat. No. C01020011

Version 2 / 21.01.14

diagenode

Content

Introduction 4

Kit method overview & time table 5

Kit Content 5

Required Materials Not Provided 5

Remarks before starting 6

Short Protocol 7

Detailed Protocol 8

STEP 1: Cell fixation and collection 8
STEP 2: Cell lysis and Chromatin shearing 9

Additional Protocols 10

Troubleshooting guide 12

Related Products 14

Ordering Information 18

Introduction

The first critical step of a successful Chromatin Immunoprecipitation (ChIP) experiment is the preparation of sheared chromatin. We therefore suggest the use one of our optimized Shearing ChIP kits.

- Chromatin Shearing Optimization kit - **Low** SDS (Cat. No. AA-001-0100)
- Chromatin Shearing Optimization kit - **Medium** SDS (Cat. No. AA-002-0100)
- Chromatin Shearing Optimization kit - **High** SDS (Cat. No. AA-003-0100)

Our Chromatin Shearing Optimization kits are used in combination with the Bioruptor® in order to ensure a highly reproducible chromatin shearing and make sure you obtain the right fragment size needed for your experiment. Establish optimal conditions required for shearing cross-linked chromatin (protein-DNA) is usually laborious; the protocol of the Chromatin Shearing Optimization kits is fast, easy-to-use and optimized to get the best results possible.

The ChIP assay offers great potential to improve knowledge about the regulation of gene expression. This technique is now used in a variety of life science disciplines including cellular differentiation, tumor suppressor gene silencing, and the effect of histone modifications on gene expression.

Isolation and shearing of the chromatin after formaldehyde fixation is the first step in the ChIP method. The following two steps are: 1/ specific immunoprecipitation of chromatin fragments using antibody against the protein of interest and 2/ analysis of the immunoprecipitated DNA fragment. With the present kit, the first step of the ChIP method is guaranteed, providing you with the best results.

Using standard protocols and kits leads to a significant decrease in potential variables that can occur from shearing to ChIP, which makes interpretation and analysis from experiment to experiment much more accessible.

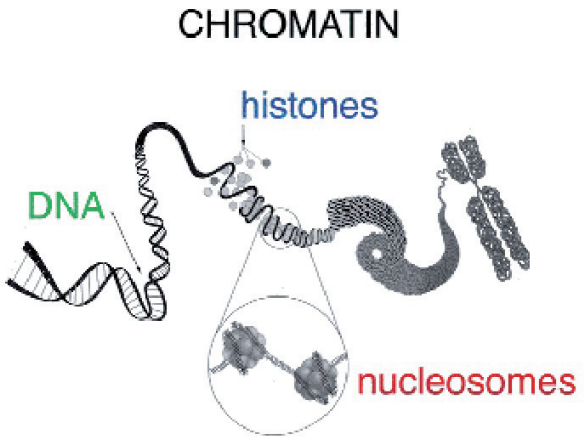


Figure 1
Representation of the chromatin

Chromatin is that portion of the cell nucleus which contains all the DNA of the nucleus in animal or plant cells. DNA in chromatin is organised in arrays of nucleosomes. Two copies of histone proteins are assembled into an octamer that has 145-147 base pairs (bp) of DNA wrapped around it to form a nucleosome core. The nucleosome, in its role as the principal packaging element of DNA within the nucleus, is the primary determinant of DNA accessibility

(Luger et al. 1997).

Kit method overview & time table

Table 1 : Protocol overview

Step		Time needed
1	Cell collection and DNA-protein crosslinking	1 to 2 hours
2	Cell lysis and Chromatin shearing	1 to 2 hours

Kit Content

Table 2: Kit content and storage

This kit contains enough reagents for the shearing of chromatin from 100 million cells. These components are identical to the ones included in the HighCell# ChIP kit (Cat. No. kch-mahigh-A16).

Description	Quantity	Storage
Glycine	2 ml	4°C
Lysis Buffer L1	25 ml	4°C
Lysis Buffer L2	25 ml	4°C
Shearing Buffer S1	5 ml	4°C

Required Materials Not Provided

Reagents

- Formaldehyde (37% stock)
- Ice-cold PBS buffer
- Agarose and TAE buffer
- DNA molecular weight marker

Equipment

- Cell scraper
- Centrifuges (at 4°C) for 1.5 ml tubes and 15 ml tubes
- Bioruptor® from Diagenode
- Agarose gel apparatus
- 30°C incubator or water bath

Remarks before starting

Before starting the ChIP, the chromatin should be sheared to fragments in the 100 to 600 bp range. Our kits and protocols are optimized for Chromatin shearing using the Bioruptor® (UCD-Standard, Plus and Twin). The maximum volume for shearing with the Bioruptor® is 300 µl per 1.5 ml microtube (depending on the specific type). We recommend using TPX tubes (Cat. No. M-50050) as shearing has been shown to be more efficient and reproducible using these tubes. The shearing conditions mentioned in the protocol are adequate for a variety of cell types.

However, given that cell types are different, we recommend optimizing sonication conditions for each cell type before processing large quantities of cells or samples. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation. A protocol to assess the shearing efficiency can be found in the "additional protocols" section.

Short protocol for experienced users

STEP 1. Cell collection and DNA-protein crosslinking

1. Use a trypsinisation method to collect the cells. Wash two times the cell suspension with PBS
2. Label new 1.5 ml tube(s), count the cells and put 1 million to 10 million cells in 500 µl of PBS.
3. Add 13.5 µl of 36.5% formaldehyde per 500 µl of sample (final concentration has to be approximatively 1%).
4. Mix by gentle vortexing. Incubate for 8 minutes at room temperature to allow fixation to take place.
5. Add 57 µl of 1.25 M Glycine to the sample.
6. Mix by gentle vortexing. Incubate for 5 minutes at room temperature. This is to stop the fixation.
   - Work on ice from this point onwards
7. Centrifuge at 1,500 rpm (300 x g) for 5 minutes at 4°C.
   - We recommend the use of a swing-out rotor with soft settings for deceleration.
8. Aspirate the supernatant. Take care to not remove the cells. Aspirate slowly and leave approximately 30 µl of the solution behind.

STEP 2. Cell lysis and chromatin shearing

9. Wash the cross-linked cells twice with 1 ml of ice-cold PBS.
10. After the last wash, aspirate the supernatant. Leave about 10 to 20 µl behind.
    - These are the cross-linked cells ready for chromatin shearing.
11. Add 1 ml of ice-cold Lysis Buffer L1 per pellet of cells. Resuspend cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing.
12. Centrifuge for 5 minutes at 1,600 rpm (500 x g) at 4°C. Discard the supernatant, keep the pellet.
13. Add 1 ml of ice-cold Lysis Buffer L2 per pellet of cells. Resuspend cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing.
14. Pellet again by centrifugation for 5 minutes at 1,600 rpm (500 x g) at 4°C, and discard supernatant.
15. Add protease inhibitor to the Shearing Buffer S1 (RT). This is the complete shearing Buffer S1 for sonication. Keep the buffer at room temperature until use; discard what is not used during the day.
16. Add 200 µl of complete Shearing Buffer S1 (RT) to the cells. Vortex until resuspention. Incubate for 10 minutes on ice.
17. Submit the samples to sonication to shear the chromatin using the Bioruptor® for two run of 10 cycles: [30 seconds "ON", 30 seconds "OFF"] each.
    - Spin and vortex between each run
    - We recommend to use TPX tubes (Cat. No. M-50050)
18. Sheared chromatin analysis step. (See additional protocols)

MANUAL

Detailed protocol

STEP 1. Cell collection and DNA-protein crosslinking

1. Pre-warm PBS, culture media and trypsin-EDTA.
2. Remove old media and rinse cells with pre-warmed PBS (Table 3). Shake dishes for 2 minutes. Remove the PBS.

Table 3			
Cell rinsing	3x 10e6 cells	10e7 cells	5x 10e7 cells
PBS	3.5 ml	10 ml	50 ml

3. Add sterile trypsin-EDTA to tissue culture flask or dish still containing adherent cells (Table 4). Brief treatment with trypsin-EDTA removes adherent cells from tissue culture flask bottom.

   Note: Each cell line will differ in the degree of adherence it has, and this will be described in the literature that accompanies it. If necessary, place flask back into incubator for about 1-2 minutes.

Table 4			
Cell detachment	3x 10e6 cells	10e7 cells	5x 10e7 cells
Trypsin-EDTA	1 ml	3 ml	15 ml

4. Check after a minute to see if cells have come off the flask bottom.

   Note: Prolonged treatment with trypsin may damage cells. This can be observed macroscopically as sheets of floating cells will be visible.

5. When cells are detached, add immediately culture medium to the cells (Table 5). The addition of medium will inactivate the trypsin.

Table 5			
Trypsin neutralisation	3x 10e6 cells	10e7 cells	5x 10e7cells
Culture medium	2 ml	6 ml	30 ml

6. Wash down sides of culture flask with a portion of the [cell/culture medium] mixture. Pipette the cells and transfer to a 15 ml or 50 ml centrifuge tube.
7. Wash two times the cell suspension with PBS.
8. Label new 1.5 ml tube(s), count the cells and put 1 million to 10 million cells in 500 µl of PBS.
   - In order to preserve the cells, use either a 1000 µl pipette tip or a smaller tip that has been cut in order to increase the opening.
9. Add 13.5 µl of 36.5% formaldehyde per 500 µl of sample (final concentration has to be approximatively 1%).
10. Mix by gentle vortexing. Incubate for 8 minutes at room temperature to allow fixation to take place.
11. Add 57 µl of 1.25 M Glycine to the sample.
12. Mix by gentle vortexing. Incubate for 5 minutes at room temperature. This is to stop the fixation.
13. Preferably work on a pre-chilled DiaMag1.5 magnetic rack or work on ice from this point onwards.
14. Centrifuge at 1,500 rpm (300 x g) for 5 minutes at 4°C.
15. We recommend the use of a swing-out rotor with soft settings for deceleration.
16. Aspirate the supernatant. Take care to not remove the cells. Aspirate slowly and leave approximately 30 µl of the solution behind.
    - These are the cross-linked cells ready for chromatin shearing.
    - Do not disturb the pellet.

STEP 2. Cell lysis and chromatin shearing

This section describes cell lysis and Bioruptor® chromatin shearing. At this stage, it is essential to produce fragments of size suitable for ChIP and subsequent PCR analysis of the immunoprecipitated DNA. The average size is of 500 base pairs (bp) (range: 200 - 1,000 bp).

Work on ice unless otherwise stated.

17. Wash the cross-linked cells twice with 1 ml of ice-cold PBS (adding NaBu (20mM final concentration) and/or any other inhibitor of choice).
    - Add the NaBu-PBS solution, gently vortex and centrifuge at 470 x g (in a swing-out rotor with soft settings for deceleration) for 10 minutes at 4 °C.
    - Resuspend with a pipette to ensure cells are thoroughly washed.
    - In any case make sure that cells are in suspension before proceeding to the next point.
18. After the last wash, aspirate the supernatant. Leave about 10 to 20 µl behind.
    - These are the cross-linked cells ready for chromatin shearing.
    - Avoid taking out too much as that could lead to material loss.
19. Add 1 ml of ice-cold Lysis Buffer L1 per pellet of cells. Resuspend cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing.
20. Centrifuge for 5 minutes at 1,600 rpm (500 x g) at 4°C. Discard the supernatant, keep the pellet.
21. Add 1 ml of ice-cold Lysis Buffer L2 per pellet of cells. Resuspend cells by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing.
22. Pellet again by centrifugation for 5 minutes at 1,600 rpm (500 x g) at 4°C, and discard supernatant.
23. Add protease inhibitor to the Shearing Buffer S1 (RT). This is the complete shearing Buffer S1 for sonication. Keep the buffer at room temperature until use; discard what is not used during the day.
24. Add 200 µl of complete Shearing Buffer S1 (RT) to the cells. Vortex until resuspention. Incubate for 10 minutes on ice.
25. Submit the samples to sonication to shear the chromatin using the Bioruptor® for two run of 10 cycles: [30 seconds "ON", 30 seconds "OFF"] each.
    - Spin and vortex between each run
    - We recommand to use TPX tubes (Cat. No. No. M-50050)
26. Sheared chromatin analysis step. (see additional protocols)

Aditional Protocols

Sheared chromatin analysis

This protocol refers to the Diagenode's Elution Module (Cat. No. mc-magme-002) that can be ordered separately.

Reagents not supplied:

- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- 100% Ethanol
- 70% Ethanol

1. Take an aliquot of 50µl of sheared chromatin and spin it at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.
2. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1µl of cocktail in 150 µl of water).
3. Add 2 µl of diluted RNase cocktail.
4. Incubate 1h at 37°C.
5. Prepare the Complete Elution Buffer by mixing thoroughly Buffers D, E and F as follows

Reagents	Volume
Buffer D	96 µl
Buffer E	10 µl
Buffer F	4 µl
Total volume	110 µl*

* enough volume for two chromatin samples

6. Add 54 µl of the Complete Elution Buffer to each chromatin sample.
7. Mix thoroughly before incubating sample at 65°C for 4h (or overnight).
8. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol. Incubate the sample at RT for 10 min on a rotating wheel.
9. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
10. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.
11. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
12. Precipitate the DNA by adding 10 µl of meDNA precipitant, 5 µl of meDNA coprecipitant and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
13. Centrifuge for 25 min at 13,000rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.
14. Centrifuge for 10 min at 13,000 rpm at 4°C. Carefully remove the supernatant, leave tubes opened for 30 min at RT to evaporate the remaining ethanol.
15. Re-suspend the pellet in 20 µl of TE buffer.
16. Run samples (20 µl of DNA + 4 µl of 6x loading dye) on a 1.5% agarose gel. As an alternative run 1 µl on a microfluidic chip (e.g. Bioanalyzer High Sensitivity DNA chip).

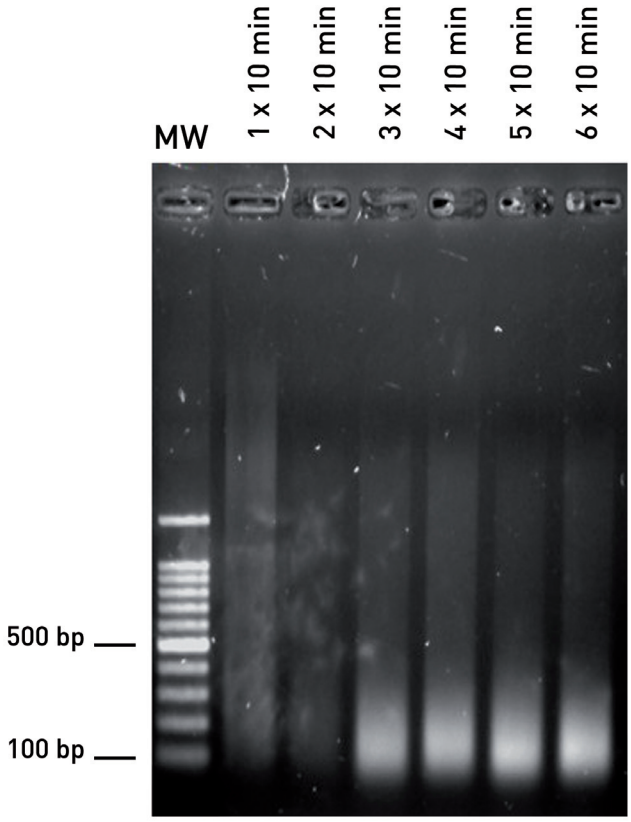


Figure 2

Time course sonication experiment with the Bioruptor® PLUS using buffers and protocol of Diagenode's Chromatin Shearing Optimization kit - Medium SDS

HeLa cells are fixed with 1% formaldehyde (for 8 min at RT). Nucleus/Nuclei isolation of 5 million cells are performed using buffers of Diagenode's Chromatin Shearing Optimization kit - Medium SDS (Cat. No. AA-002-0100) and are then resuspended in 200 µl of Shearing Buffer S1 prior to Chromatin shearing.

Samples are sheared during 1, 2, 3, 4, 5 or 6 rounds of 10 cycles of 30 sec ON/30 sec OFF with the Bioruptor® PLUS combined with the Bioruptor® Water cooler (Cat. No. BioAcc-cool) & Single Cycle Valve (Cat. No. VB-100-0001) at HIGH power setting (position H). All samples were treated with RNase (see **Additional protocols**) prior to agarose gel analysis. 300 ng of each sample was analyzed on a 1.5% agarose gel (except for lanes 2 and 3).

In this example, the optimal shearing condition (the least time course condition) corresponds to 3 rounds of 10 cycles (30 sec ON/30 sec OFF).

Troubleshooting guide

Process	Protocol step	Issues and resolutions
Crosslinking and fixation	Optimize crosslinking time	Poor crosslinking causes DNA loss, elevated background, and/or reduced antigen availability in chromatin. Emperically determine optimal crosslinking time for maximal specificity and efficiency of ChIP. The optimal duration of cross-linking varies between cell type and protein of interest. Short crosslinking time (5-10 minutes) may improve shearing efficiency. Crosslinking duration should not exceed 30 minutes or shearing will be inefficient.
	Assure proper fixation time with formaldehyde	Crosslinking may be too weak or too strong without proper fixation time. Optimize fixation step e.g: incubate for 8 minutes at room temperature with high-quality, fresh 1% formaldehyde final concentration (weight/volume).
	Optimize formaldehyde concentration	Lower formaldehyde concentrations (1%weight/volume) may improve shearing efficiency. For some proteins, however, especially those that do not directly bind DNA, this might reduce crosslinking efficiency and thus the yield of precipitated chromatin. Empirically determine the formaldehyde concentration as some antigen epitopes may be more sensitive to formaldehyde.
Cell lysis	Make sure cells disrupt completely	Do not use too many cells per amount of lysis buffer (w/v) so that cells can be completely disrupted. Follow the instructions in the protocol (e.g.: 1 million cells or less/130 µl of complete Buffer B). See steps 2 and 3.
	Maintain cold temperature during lysis	Perform cell lysis at 4°C (cold room) or on ice. Always keep the samples ice cold during cell lysis and use cold buffers as in Step 3.
	Prevent protein degradation	Add the protease inhibitors to the lysis buffer immediately before use.
Cell type	Determine which cell types have previously been validated with the kit	HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells), U2OS and kerationocytes have been used to validate this magnetic ChIP protocol.
Number of cells required	Determine number of cells for ChIP	The number of cells for ChIP is determined by cell type, protein of interest, and antibodies used. Use chromatin from 1,000 to 10,000 cells per ChIP. (In some cases, chromatin from up to 100,000 cells may be needed). You may need to empirically determine the optimal number.
Chromatin shearing	Maintain 4°C temperature during shearing	Keep samples cold at 4°C before sonication to maintain sample integrity.
	Maintain 0ÐC temperature during sonication	Maintain temperature of the samples at 4°C to maintain sample integrity.
	Optimize SDS concentration	High % SDS favours better sonication but inhibits immunoselection (optimal range: 0.1% to 1%). Final SDS concentration should not be higher than 0.15 to 0.20% (e.g. If the shearing buffer contains 0.75% SDS, the sheared chromatin is diluted 3.5 to 4.0 fold in the ChIP buffer.
	Determine amount of sheared chromatin needed for ChIP	Most of the sheared chromatin will be used for ChIP and the input control. A small amount will be checked on agarose gel.
	Dilute the sheared chromatin in ChIP buffer for Immunoselection incubation	The sheared chromatin is diluted in complete Buffer A prior to the immunoselection incubation (see Step 3). Dilute the sheared chromatin at least 7-fold. Adjust the ChIP buffer volume added to the chromatin accordingly.

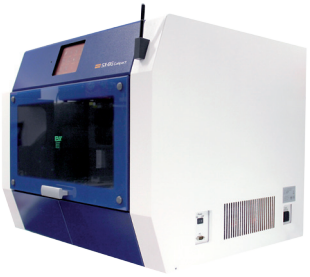
Sonication tips	Determine cell number	Start with 1x10e6 cells or less.
	Sonication conditions with the Bioruptor®	Shear the samples of chromatin using the Bioruptor® for 10-12 cycles of: [30 seconds "ON" / 30 seconds "OFF"] each. These conditions were tested with many mammalian cell lines and were excellent for subsequent ChIP experiments. A troubleshooting guide for Bioruptor-chromatin shearing is available.
	Chromatin shearing with Diagenode modules	You can also use the other Chromatin Shearing Optimization kits which differ by their SDS concentration (Cat. No. AA-001-0100 ; AA-002-0100)
Gel analysis of sheared chromatin	Load enough DNA on gel	Chromatin equivalent to at least 100,000 cells can be be visualized on a gel. Do not use an excessive amount or it will obscure the visualization. The DNA amount to load depends on well size and on the gel size.
	Use correct agarose concentration	Use a 1-1.5% agarose gel.
	Use correct running buffer concentration and run time	1x TAE or TBE is preferred to 0.5x TAE (which can lead to smears). Run slowly.

Related Products

Automated ChIP kits			
	Auto ChIP kit (1)	**Auto Histone ChIP-seq kit (1)**	**Auto Transcription ChIP kit (1)**
Features	All DNA-protein interaction, saving time, maximum reproducibility	Optimized for working with histone antibodies in ChIP-seq experiments, saving time, maximum reproducibility	Optimized for working with TF anbosies, saving time, maximum reproducibility
Optimized for	All DNA-protein interactions	Histones and histone modifications	Transcription factors and co-factors
Downstream applications	qPCR	qPCR, sequencing, arrays (2)	qPCR, sequencing, arrays (2)
Amount of cells/IP	1.000 - 1 million	1.000 - 10 million	1.000 - 10 million
Total Time of Assay	1 day	1 day	1 day
Handling time	30 min	30 min	30 min
Buffers and reagents	IP, DNA Isolation	IP	IP
Control antibodies	anti-IgG (rabbit)	anti-IgG (rabbit)	anti-IgG (rabbit)
DNA purification	DNA isolation buffer (DIB)	-	-
#rxns per kit	16 or 100	16 or 100	16 or 100
Cat. No.	AB-Auto01-A016 AB-Auto01-G016 AB-Auto01-A100 AB-Auto01-G100	AB-Auto02-A016 AB-Auto02-G016 AB-Auto02-A100 AB-Auto02-G100	AB-Auto03-A016 AB-Auto03-G016 AB-Auto03-A100 AB-Auto03-G100

(1) Validated on SX-8G IP-Star® and SX-8G IP-Star® Compact Automated Systems

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



Manual ChIP kits			
	iDeal ChIP-seq kit	**LowCell# ChIP kit**	**HighCell# ChIP kit**
Features	Only kit on the market validated for ChIP-seq on GAIIx (Illumina®) and PGM™ (Ion Torrent™). Providing high signal and minimum background in sequencing	Magnetic bead-based protocol for all DNA-protein interaction, fast, increased DNA yield	Magnetic bead-based protocol Ideal to recover large amount of DNA (transcription factors, ChIP-on-chip) and to avoid biais due to amplification steps
Optimized for	Histones and histone modifications	All DNA-protein interactions	All DNA-protein interactions
Suitable for ChIP-seq and ChIP-on-chip	Yes	Yes (1)	Yes (1)
Amount of cells/IP	1 million cells	1.000 - 1 million	1 - 10 million
Total Time of Assay	1 day	1 day	1 day
Handling time	1.5 h	1.5 h	1.5 h
Buffers and reagents for	Cell Lysis, chromatin shearing, IP, DNA purification (IPure)	Cell lysis, chromatin shearing, IP, DNA purification	Cell lysis, chromatin shearing, IP, DNA purification
Control antibodies	Rabbit IgG, H3K4me3 antibody	anti-IgG (rabbit)	anti-IgG (rabbit)
Control PCR primer pairs	ChIP-seq grade GAPDH TSS primer pair, ChIP-seq grade Myoglobin exon 2 primer pair	human TSH2B / c-fos / myoglobin exon 2	human TSH2B / GAPDH promoter
DNA purification	IPure kit	DNA isolation buffer	DNA isolation buffer
#rxns per kit	24	16 or 48	16
Cat. No.	AB-001-0024	kch-maglow-A16 kch-maglow-G16 kch-maglow-A48 kch-maglow-G48	kch-mahigh-A16 kch-mahigh-G16

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

DNA methylation kits

Automated DNA methylation kits			
	Auto MethylCap kit (1)	**Auto MeDIP kit (1)**	**Auto hMeDIP kit (1)**
Features	Allows to capture fractions of methylated DNA by CpG density. Includes control primer pairs for assessment of capture efficiency	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-meC Ab), fast	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-hmC Ab), fast
Downstream applications	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).	qPCR, linear amplification and genome-wide analysis such as microarray and sequencing (2).
Amount of DNA/rxn	1 µg	1 µg	1 µg
Total Time of Assay	1 day	1 day	1 day
Handling time	30 min	30 min	30 min
Internal controls	-	Methylated and unmethylated BAC clones	Hydroxymethylated, methylated and unmethylated BAC clones
Control PCR primer pairs	TSH2B/GAPDH	Methylated DNA control unmethylated DNA control	Hydroxymethylated DNA control methylated DNA control unmethylated DNA control Sfi1 for genomic DNA
#rxns per kit	48	16 or 100	16
Cat. No.	AF-Auto01-0048	AF-Auto01-A016 AF-Auto01-G016 AF-Auto01-A100 AF-Auto01-G100	AF-Auto02-0016

(1) Validated on SX-8G IP-Star® and SX-8G IP-Star® Compact Automated Systems

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

Manual DNA methylation kits					
	MethylCap kit	**MagMeDIP kit**	**MeDIP kit**	**hMeDIP kit**	**MagBisulfite kit**
Features	Allows to capture fractions 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, 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	Quality control using internal controls, improved handling (magnetic beads), high specificity (monoclonal 5-hmC Ab), fast	Gives precise information on methylation status of single cytosines. High conversion rate →99%. DNA purification based on magnetic beads and compatible with SX-8G IP-Star® Automated System.
Suitable for	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, linear amplification and genome-wide analysis (microarray, sequencing). Depending on the DNA purification method selected	qPCR, sequencing, microarray
Amount of DNA/ rxn	1 µg	1 µg	1 µg	1 µg	1 ng - 1 µg
Total Time of Assay	1 day	2 or 3 days	2 or 3 days	2 or 3 days	3.5 hours
Handling time	2h	1.5 h	2h	1.5 h	2h
Internal controls	-	Methylated and unmethylated BAC clones	Methylated and unmethylated BAC clones	Hydroxymethylated, methylated and unmethylated BAC clones	-
Control PCR primer pairs	TSH2B/GAPDH	Methylated DNA control unmethylated DNA control TSH2B GAPDH	Methylated DNA control unmethylated DNA control TSH2B GAPDH	Hydroxymethylated DNA control / methylated DNA control / unmethylated DNA control / Sfi1 for genomic DNA	Bisulfite-specific primer pair
#rxns per kit	48	10 or 48	10	16	24
Cat. No.	AF-100-0048	mc-magme-A10 mc-magme-048	mc-green-003	AF-104-0016 AF-110-0016 AF-111-0016	AF-106-0024

Ordering information

Diagenode s.a. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite #101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

For a complete listing of Diagenode's international distributors visit:
http://www.diagenode.com/en/company/distributors.php
For rest of the world, please contact Diagenode s.a.

FOR RESEARCH USE ONLY.
Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER

LIMITED LICENSE

The information provided herein is owned by Diagenode and/or its affiliates. Subject to the terms and conditions that govern your use of such products and information, Diagenode and/or its affiliates grant you a nonexclusive, nontransferable, non-sublicensable license to use such products and information only in accordance with the manuals and written instructions provided by Diagenode and/or its affiliates. You understand and agree that except as expressly set forth in the terms and conditions governing your use of such products, that no right or license to any patent or other intellectual property owned or licensable by Diagenode and/or its affiliates is conveyed or implied by providing these products. In particular, no right or license is conveyed or implied to use these products in combination with any product not provided or licensed to you by Diagenode and/or its affiliates for such use.

Limited Use Label License: Research Use Only The purchase of this product conveys to the purchaser the limited, non-transferable right to use the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact info@diagenode.com.

TRADEMARKS

The trademarks mentioned herein are the property of Diagenode or their respective owners. Bioruptor is a registered trademark of Diagenode SA.

Bioanalyzer is a trademark of Agilent Technologies, Inc.

MA-CSh-MEDIUM-SDS-V2-21_01_14

orders@diagenode.com
orders.na@diagenode.com
www.diagenode.com

diagenode