diagenode

A Hologic Company

Auto ChIPmentation for Histones

Cat. No. C01011000

USER GUIDE

Version 2 01_03_2018

Please read this manual carefully
before starting your experiment

Contents

Introduction

Association between proteins and DNA influences various vital cellular functions such as cellular proliferation and epigenetic regulation. It is therefore crucial to understand these interactions and the mechanisms by which they control and guide gene expression. Chromatin Immunoprecipitation (ChIP) is a technique to analyze the association of proteins with specific genomic regions in intact cells, by using a specific antibody that targets the protein of interest. ChIP can be used to study changes in epigenetic signatures, chromatin remodeling and transcription regulator recruitment to specific genomic sites.

In addition, Next-Generation Sequencing (NGS) makes it possible to analyze DNA-protein interactions at a genome-wide level, but requires many additional processing steps of the samples in order to generate libraries with sequencing adaptors.

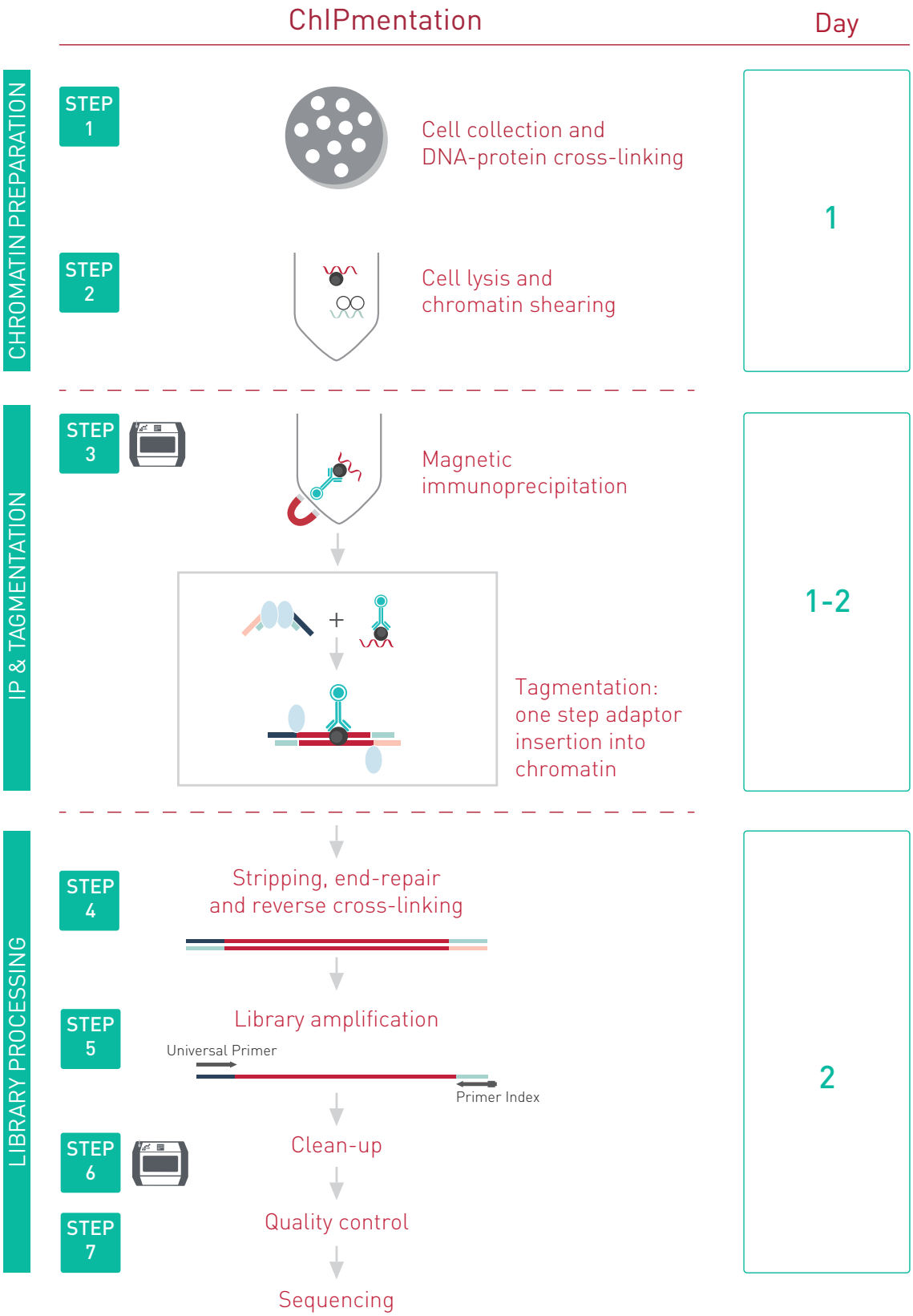
In traditional ChIP-seq library preparation, the ligation of adaptors is performed after chromatin immunoprecipitation and prior to library preparation. This process requires three additional steps that include the adaptor ligation that may introduce artifacts and the need for greater hands-on time.

The new Diagenode ChIPmentation technology solves this problem. ChIPmentation is based on tagmentation which enables the integration of the library preparation during the ChIP itself using transposase and sequencing-compatible adaptors. In addition, Diagenode's ChIPmentation automates the main steps of chromatin immunoprecipitation and library preparation on the Diagenode IP-Star®. Unlike standard library preparation techniques that require multi-step ligation, ChIPmentation incorporates a much easier and shorter protocol. The combination of direct adaptor incorporation and automation allows for higher sensitivity from low cell numbers and reproducibility of results.

Benefits of the ChIPmentation system for optimal ChIP-seq

- Automate on the IP-Star® to support standardization and reproducibility
- Ensure high quality data on low cell numbers and rare cell types
- Save one full day from standard protocols in generating ChIP-seq libraries
- Benefit from the elimination of sequencing adaptor dimers
- Enjoy an easier protocol

Kit method overview & time table



LEGEND

Protein of interest
Other protein
DNA
Antibody
Magnetic bead
Magnet
Tagmentation Enzyme loaded with adaptors
IP-Star

Kit materials

The Auto ChIPmentation for Histones contains all reagents necessary for chromatin preparation, chromatin immunoprecipitation and library preparation for NGS. The kit allows the preparation of 4 chromatin preparations (up to 10 M cells per batch), 24 ChIP reactions, 4 inputs and 24 libraries. Reagents for chromatin size assessment of each batch of chromatin are also included.

Components supplied with the ChIPmentation for histones

NOTE: Upon receipt, store the components at the indicated temperatures.

Table 1. Components for ChIP (iDeal ChIP-seq kit for Histones)

Component	Volume	Storage
Protease inhibitor cocktail	90 µl	-20°C
5% BSA (DNA free)	175 µl	-20°C
Rabbit IgG	8 µg	-20°C
ChIP-seq grade antibody H3K4me3	8 µg	-20°C
ChIP-seq grade GAPDH TSS primer pair	96 µl	-20°C
ChIP-seq grade Myoglobin exon 2 primer pair	96 µl	-20°C
Carrier	64 µl	-20°C
Glycine	480 µl	4°C
Shearing Buffer iS1	8 ml	4°C
DiaMag protein A-coated magnetic beads	480 µl	4°C
Wash buffer iW1	10 ml	4°C
Wash buffer iW2	10 ml	4°C
Wash buffer iW3	10 ml	4°C
Wash buffer iW4*	10 ml	4°C
ChIP-seq grade water	10 ml	4°C
Elution Buffer iE2	175 µl	4°C
Lysis Buffer iL1	44 ml	4°C
Lysis Buffer iL2	44 ml	4°C
Wash buffer 1 w/o iso-propanol	2 ml	4°C
Wash buffer 2 w/o iso-propanol	2 ml	4°C
Buffer C	2 ml	4°C
IPure Beads v2	320 µl	4°C
Elution Buffer iE1	4 ml	4°C
5x ChIP Buffer iC1	4 ml	4°C

*This reagent is not used in the ChIPmentation protocol.

Table 2. Components for library preparation

Component	Volume	Storage
Tagmentation enzyme	24 µl	-20°C
PCR MasterMix	820 µl	-20°C
100 x SYBR	3 µl	-20°C
Universal primer	44 µl	-20°C
Primer Index 1	12 µl	-20°C
Primer Index 2	4,5 µl	-20°C
Primer Index 3	4,5 µl	-20°C
Primer Index 4	4,5 µl	-20°C
Primer Index 5	4,5 µl	-20°C
Primer Index 6	4,5 µl	-20°C
Primer Index 7	4,5 µl	-20°C
Primer Index 8	4,5 µl	-20°C
Primer Index 9	4,5 µl	-20°C
Primer Index 10	4,5 µl	-20°C
Primer Index 11	4,5 µl	-20°C
Primer Index 12	4,5 µl	-20°C
Primer Index 13	4,5 µl	-20°C
Primer Index 14	4,5 µl	-20°C
Primer Index 15	4,5 µl	-20°C
Primer Index 16	4,5 µl	-20°C
Primer Index 17	4,5 µl	-20°C
Primer Index 18	4,5 µl	-20°C
Primer Index 19	4,5 µl	-20°C
Primer Index 20	4,5 µl	-20°C
Primer Index 21	4,5 µl	-20°C
Primer Index 22	4,5 µl	-20°C
Primer Index 23	4,5 µl	-20°C
Primer Index 24	4,5 µl	-20°C
Tagmentation Buffer	870 µl	4°C
Wash Buffer tagW1	13,2 ml	4°C
Wash Buffer tagW2	9,6 ml	4°C
Stripping Reagent	300 µl	4°C
$MgCl_2$	300 µl	4°C
Nuclease-free water	200 µl	4°C
Resuspension Buffer	500 µl	4°C

Indexes

Table 3. List of the 24 primer indexes with their indexes sequences

Reagent	Index Sequence
Primer Index 1	TAAGGCGA
Primer Index 2	CGTACTAG
Primer Index 3	AGGCAGAA
Primer Index 4	TCCTGAGC
Primer Index 5	GGACTCCT
Primer Index 6	TAGGCATG
Primer Index 7	CTCTCTAC
Primer Index 8	CAGAGAGG
Primer Index 9	GCTACGCT
Primer Index 10	CGAGGCTG
Primer Index 11	AAGAGGCA
Primer Index 12	GTAGAGGA

Reagent	Index Sequence
Primer Index 13	GTCGTGAT
Primer Index 14	ACCACTGT
Primer Index 15	TGGATCTG
Primer Index 16	CCGTTTGT
Primer Index 17	TGCTGGGT
Primer Index 18	GAGGGGTT
Primer Index 19	AGGTTGGG
Primer Index 20	GTGTGGTG
Primer Index 21	TGGGTTTC
Primer Index 22	TGGTCACA
Primer Index 23	TTGACCCT
Primer Index 24	CCACTCCT

With the listed index primers above, we recommend the following combinations if the multiplexing of libraries is required:

Multiplexing level	Index combination
2 samples	Index 1 - Index 2 Index 2 - Index 4
3 samples	Index 1 - Index 2 - Index 4 Index 3 - Index 5 - Index 6 Index 10 - Index 11 - Index 12 Index 13 - Index 14 - Index 22 2-plex option with any other index
4 samples	Index 8 - Index 9 - Index 11 - Index 12 Index 8 - Index 12 - Index 21 - Index 24 Index 1 - Index 2 - Index 3 - Index 4 Index 4 - Index 7 - Index 19 - Index 23 3-plex option with any other index

For 5 - 11-plex pools use 4-plex options with any other available indexes. If a higher multiplexing degree is required (>= 12), any combination is possible regardless of the index chosen.

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- RNase/DNase-free 1.5 ml tubes
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS) buffer
- qPCR SYBR® Green Mastermix
- ChIP-seq grade antibodies – www.diagenode.com
- Fluorescence-based assay for DNA concentration measurement, e.g. the Qubit High Sensitivity assay (Life Technologies #Q32851)
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- 100% Ethanol, Molecular Grade

Equipment

- Cell counter system
- IP-Star® Compact Automated System (Diagenode, Cat. No. B03000002)
- Bioruptor® sonication device and the associated microtubes:
    - Bioruptor® Pico (Diagenode, Cat. No. B01060001) and 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
    - Bioruptor® Plus (Diagenode, Cat. No. B01020001) and 1.5 ml TPX Microtubes (Cat. No. C30010010) or
- Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
- DiaMag Rotator (Rotating wheel) (Diagenode, Cat. No. B05000001)
- Vortex
- Heating block
- Qubit® Fluorometer (ThermoFisher Scientific)
- qPCR cycler
- Thermocycler

- Magnetic rack for 0.2ml tubes: DiaMag02 (Diagenode, Cat. No. B04000001) and IP-Star® strips
- Sizing equipment such as BioAnalyzer (Agilent) or Fragment Analyzer (Advanced Analytical) and their associated high sensitivity kits.

Plastics and consumables for IP-Star®

Description	Reference
200 µl tube strips (8 tubes/strip) + cap strips for SX-8G IP-Star® Compact	C30020002
96 well microplates for IP-Star®	C30080030
Tips (box)	C30040021
Tips (bulk)	C30040020
2 ml microtube for SX-8G IP-Star® Compact	C30010014
Medium reagent container for SX-8G IP-Star® Compact	C30020003

Optional supplies

- Chromatin Shearing Optimization kit - Low SDS (iDeal Kit for Histones) (Cat. No. C01020010)
- 1M Sodium butyrate (NaBu) (Diagenode, Cat. No. C12020010)
- RNase cocktail (e.g. Ambion, AM2286A), required for chromatin shearing assessment

Remarks before starting

1. Cell number

The protocol describes the preparation of a batch of chromatin from approximately 7 million cells which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 1 million cells per IP reaction are used in this standard protocol. The protocol is optimized for the use of 100 µl of sheared chromatin and it is crucial to keep these volumes consistent for optimal results.

ChIPmentation enables working with a low number of cells. Depending on the abundance of the target, the specificity of the antibody, and the number of cells available, the protocol can be adjusted, following one of those two options:

A. For using lower numbers of cells per IP, you can start with a batch of 7 million of cells (as in a standard protocol) and follow the protocol up to the chromatin shearing step. Then simply dilute the sheared chromatin in shearing buffer iS1 before adding it to the IP reaction. The final volume of diluted chromatin containing the desired number of cells should be 100 µl per IP reaction.

B. If starting with a number of cells that is different from the standard protocol , first determine the number of cells that you will use per IP and the total number of IPs. Fix cells as described in the standard protocol. For cell collection and lysis, scale up or down the volume of iL1 and iL2 buffers using 1 ml of iL1 and 1 ml of iL2 per 1 million cells. Define the volume of shearing buffer iS1 taking into account that you will need:

- 100 µl of sheared chromatin (containing the desired number of cells) per IP reaction
- 1 µl of sheared chromatin per input
- 50 µl of sheared chromatin for chromatin shearing assessment
- 5% excess of iS1 (must be added)

Resuspend the cells in the required volume of shearing buffer iS1 and follow the standard protocol.

Please note that an increased or decreased cell concentration in the shearing buffer may impact the shearing efficiency and an additional optimization of the shearing conditions may be required.

2. Fixation optimization

Formaldehyde is the most commonly used cross-linking reagent ideal for two molecules which interact directly. The fixation time can depend on your target of interest and might require additional optimization (usually a fixation of 8 to 10 minutes is suitable for most histone proteins). Please note that a longer fixation may lead to chromatin that is resistant to sonication.

3. Shearing optimization

Chromatin shearing is one of the most critical steps for a successful ChIP experiment. Chromatin fragments between 100-600 bp are ideal for the ChIP experiments. The optimal time of sonication depends on many factors such as cell type, cell density, sample volume, fixation time, etc. Hence it is important to optimize the sonication conditions for each new ChIP project. For optimization of the shearing conditions, we recommend using the Chromatin Shearing Optimization kit - Low SDS (iDeal Kit for Histones) (Cat. No. C01020010) which contains all buffers needed for chromatin preparation compatible with the Auto ChIPmentation for Histones Kit. The reagents included in this kit allow preparing chromatin using already optimized shearing settings only; it does not contain sufficient reagents for optimization of chromatin shearing.

When using the Bioruptor® Pico, an initial time-course experiment of 5-10-15 sonication cycles 30'' ON/30'' OFF is recommended. Please refer to the Guide for successful chromatin preparation using the Bioruptor® Pico (https://www.diagenode.com/files/protocols/bioruptor-pico-chromatin-preparation-guide.pdf)

When using the Bioruptor® Plus, an initial time-course experiment of 10-20-30 sonication cycles 30'' ON/30'' OFF at High Power is recommended.

Please refer to The Ultimate Guide for Chromatin Shearing Optimization with Bioruptor® Standard and Plus (https://www.diagenode.com/files/protocols/The_Ultimate_Guide_for_Chromatin_Shearing_Optimization_with_Bioruptor_protocol.pdf)

Choose the shortest sonication time resulting in an efficient chromatin shearing. Avoid over-sonication, as it may lead to a drop in efficiency in ChIP experiments.

4. Magnetic beads

This kit includes DiaMag Protein A-coated magnetic beads. Make sure the beads do not dry out during the procedure as this will result in reduced performance. Keep the beads homogenous in suspension at all times when pipetting. Variation in the amount of beads will decrease reproducibility. Do not freeze the beads.

DiaMag Protein A-coated magnetic beads are suitable for immunoprecipitation of rabbit polyclonal antibodies, mouse IgG2a, IgG2b and IgA, guinea pig IgG, dog IgG, pig IgG.

5. ChIP-seq grade antibodies

The quality of antibodies used in ChIP-seq is essential for success. It is recommended to use only validated antibodies that specifically recognize the target. Diagenode offers extensively validated and high-performance antibodies, confirmed for their specificity in ChIP-seq. Each batch is validated, and batch-specific data are available on the website www.diagenode.com

6. Input

The input sample corresponds to entirety of the DNA that undergoes the full ChIP procedure without any immunoselection. The input sample is used as a reference to calculate the recovery by qPCR at the end of the ChIP procedure. We recommend including one input for each series of ChIP reactions. The input is a good control for qPCR, however it is not incubated with the Tagmentation enzyme and therefore it cannot be sequenced.

7. Negative and positive controls

The kit contains a negative (IgG) and a positive (H3K4me3) control antibody to monitor the efficiency of the IP on the same chromatin as the one used with the antibody of interest. We recommend including one negative IgG control in each series of ChIP reactions because it can also be used by most of the bioinformatics tools for analysis of ChIP-seq data where it serves to determine the bias which may result from experimental conditions. We also recommend using the positive control ChIP-seq grade H3K4me3 antibody at least once.

8. Tagmentation time

The optimal tagmentation time can vary between 1 and 30 minutes depending on several factors like the abundance of the target, the cell number and the affinity of the antibody for the target. The recommended 10 minutes have been validated on multiple histone marks and cell numbers, therefore it should be suitable for a large range of conditions. Nevertheless if the Ct values obtained at step 5.6 are too high, resulting in a needed number of amplification cycles superior to 17, the tagmentation time may be increased in order to improve the library preparation efficiency. At the opposite, if the percentage of recovery after the immunoprecipitation is not satisfying the tagmentation time may be decreased in order to increase signal-to-noise.

9. Quantification

After ChIPmentation, determine the concentration of the libraries with a highly sensitive method such as the dsDNA HS Assay Kit on the Qubit® system from ThermoFisher Scientific. PicoGreen® is also suitable but UV spectrophotometric methods such as the NanoDrop are usually not sufficiently sensitive.

10. Quantitative PCR analysis

Prior to the sequencing, we recommend analysing the input and immunoprecipitated samples by SYBR® Green qPCR using at least 1 positive and 1 negative control region to determine the enrichment. The kit contains two primer pairs targeting two regions which are positive

(GAPDH TSS control region) and negative (Myoglobin Exon 2) for the control antibody provided in the kit (H3K4me3 grade antibody). Each specific antibody will require specific control primers designed by the user. For each primer pair, run the input DNA alongside the immunoprecipitated samples. You can dilute the DNA to perform sufficient PCR reactions. PCR reactions should be performed at least in duplicate although performing in triplicate is recommended to be able to identify potential outliers.

PROTOCOL

AUTO CHIPMENTATION PROTOCOL

STEP 1

Cell collection and DNA-protein cross-linking from cultured cells

 *30 minutes*

For adherent cells:

1.1. Pre-warm **PBS**, culture medium and **trypsin-EDTA** at 37°C.

NOTE: Cold PBS will be needed at step 1.12.

1.2. Remove the medium and rinse the cells with **pre-warmed PBS** (10 ml for a 75 cm^2 culture flask). Gently shake the flask for 2 minutes.

1.3. Remove the PBS and add **sterile trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the cells. Gently shake the culture flask for 1-2 minutes or until the cells start to detach. The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment with trypsin may damage the cells. Regularly check if the cells start to detach.

1.4. Immediately add **fresh culture medium** to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 50 ml tube.

1.5. Rinse the flask by adding **10 ml of PBS**. Add this volume to your 50 ml tubes containing cells from point 1.4. Proceed immediately with step 1.6.

For suspension cells:

Collect suspension cells in a 50 ml tube and go directly to point 1.6 of the protocol.

1.6. Centrifuge for 5 minutes at 500 x g and 4°C and remove the supernatant.

1.7. Resuspend the cells in **20 ml of PBS** and count them. Collect the cells by centrifugation for 5 minutes at 500 x g and 4°C.

1.8. Resuspend the cells in **PBS** to obtain a concentration of **7 million cells per 500 µl of PBS**. Label 1.5 ml tubes and aliquot 500 µl of cell suspension in each tube.

NOTE: If desired, the cell concentration can be adjusted from 1 to 10 million cells per 500 µl.

1.9. Under a fume hood, add **13.5 µl of 37% formaldehyde** to each tube containing 500 µl of cell suspension. Mix by gentle vortexing and incubate 8 minutes at room temperature to allow fixation to take place.

NOTE: The fixation time might require an additional optimization. Please refer to the "Remarks before starting".

1.10. Add **57 µl of Glycine** to the cells to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking. Keep everything at 4°C or on ice from now on.

1.11. Collect the cells by centrifugation at 500 x g for 5 minutes and 4°C. Discard the supernatant without disturbing the cell pellet.

1.12. Wash the cells twice with **1 ml of cold PBS** as follows:
Add cold PBS
Invert the tube 2-3 times
Centrifuge at 500 x g 5 minutes at 4°C

NOTE: We strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP. If not possible, the pellets of fixed cells can be stored at -80°C for up to 4 months.

STEP 2

Cell lysis and chromatin shearing from cells

1 to 2 hours

2.1. Add **1 ml of ice-cold Lysis buffer iL1** to the 1.5 ml tube containing 7 million cells. Resuspend the cells by pipetting up and down several times and transfer them to a 15 ml tube. Add 6 ml of buffer iL1 and incubate for 10 minutes at 4°C with gentle mixing.

NOTE: For scaling up or down, use 1 ml of buffer iL1 per 1 million of cells.

2.2. Pellet the cells by centrifugation at 500 x g and 4°C for 5 minutes and discard the supernatant.

2.3. Resuspend the cell pellet in **1 ml of ice-cold Lysis buffer iL2** by pipetting up and down several times. Add another **6 ml of buffer iL2** and incubate for 10 minutes at 4°C with gentle mixing.

NOTE: For scaling up or down, use 1 ml of buffer iL2 per 1 million of cells.

2.4. Pellet the cells again by centrifugation for 5 minutes at 500 x g and 4°C and discard supernatant.

2.5. Prepare complete Shearing buffer by adding 3.5 µl of **200x protease inhibitor cocktail to 700 µl of Shearing buffer iS1**. Keep on ice.

NOTE: For scaling up or down, prepare 100 µl of complete Shearing Buffer per 1 million cells.

2.6. Add **700 µl of complete Shearing buffer iS1** to 7 million cells. The final cell concentration in the shearing buffer should be 1 million of cells per 100 µl of iS1. Resuspend the cells by pipetting up and down several times. Split the cell suspension into aliquots of maximum 300 µl by transferring it to the appropriate sonication microtubes:

- When using the Bioruptor® Pico use1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
- When using the Bioruptor® Plus use1.5 ml TPX Microtubes (Cat. No. C30010010)

NOTE: The maximum volume for shearing with the Bioruptor® is 300 µl per 1.5 ml Microtube.The use of correct type of microtubes is essential for the efficiency of sonication.

2.7. Shear the chromatin by sonication using the Bioruptor®. Choose the protocol which is adapted to your device:

- When using the Bioruptor® Pico shear for 5 to 12 cycles [30 seconds "ON", 30 seconds "OFF"].
- When using the Bioruptor® Standard or Plus, shear for 10 to 30 cycles [30 seconds "ON", 30 seconds "OFF"] each at High power setting.

NOTE: We recommend performing pilot experiments for each new sample type using the Chromatin Shearing Optimization kit - Low SDS (iDeal Kit for Histones) (Cat. No. C01020010).

2.8. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Pool the supernatants which contain the sheared chromatin. Use the chromatin for immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months.

2.9. Take an aliquot of 50 µl of sheared chromatin for the shearing assessment. The protocol is described in the "Additional Protocols" section.

NOTE: We recommend analysing the shearing for each batch of chromatin. This step can be omitted when optimal sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimized previously. Store the chromatin aliquot at -20°C until analysis.

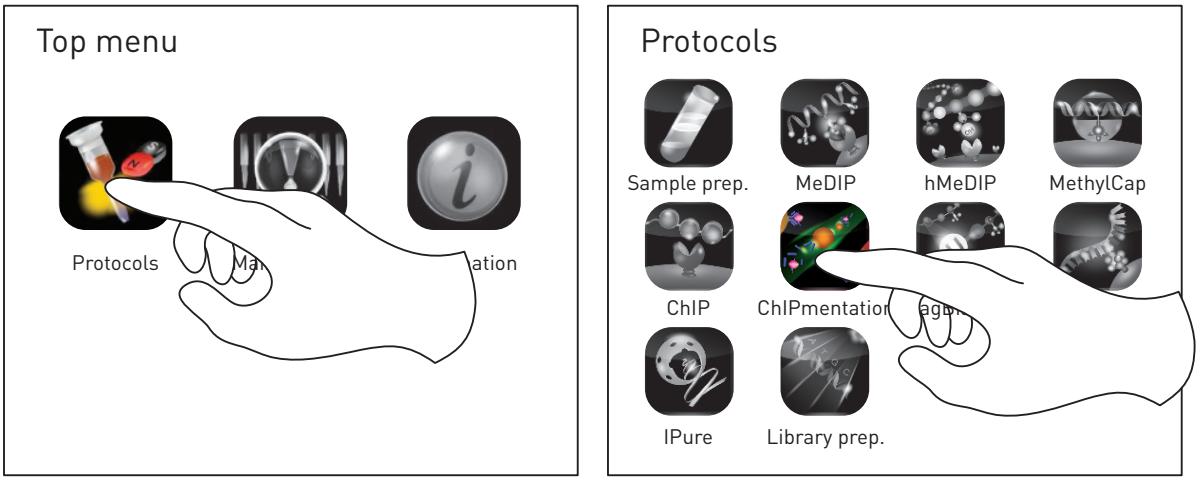
STEP 3

Magnetic immunoprecipitation and tagmentation

1.5 hours hands on time and overnight run

3.1. Switch ON the IP-Star® Compact.

3.2. Select "**Protocols**" icon and then "**ChIPmentation**" category.



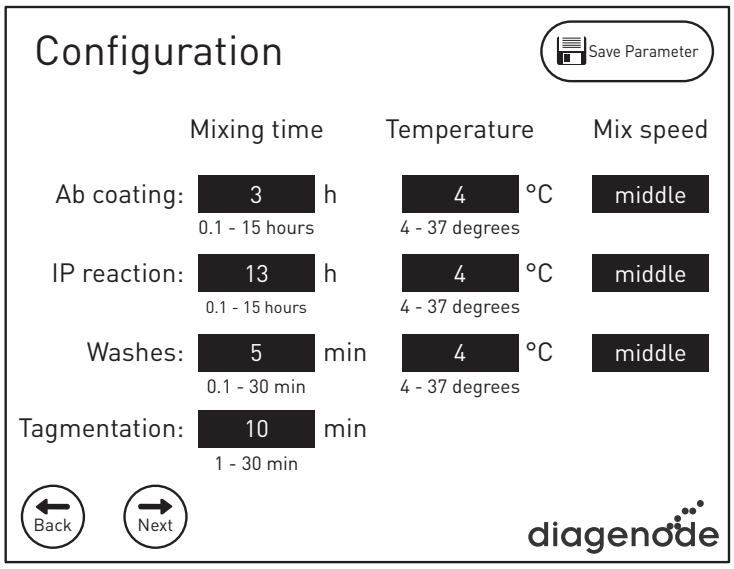
3.3. Select "**ChIPmentation_08_D**" if you plan to run between 1 and 8 samples or "**ChIPmentation_16_D**" if you plan to run between 9 and 16 samples.

3.4. Setup the exact number of samples for your experiment by pressing the black box. This number includes the positive and negative control IPs and each IP has to be counted as a sample. Input will not undergo immunoprecipitation in the IP-Star and is therefore not considered as a sample.

NOTE: The Peltier block is now cooling down to 4°C to keep your samples cold.

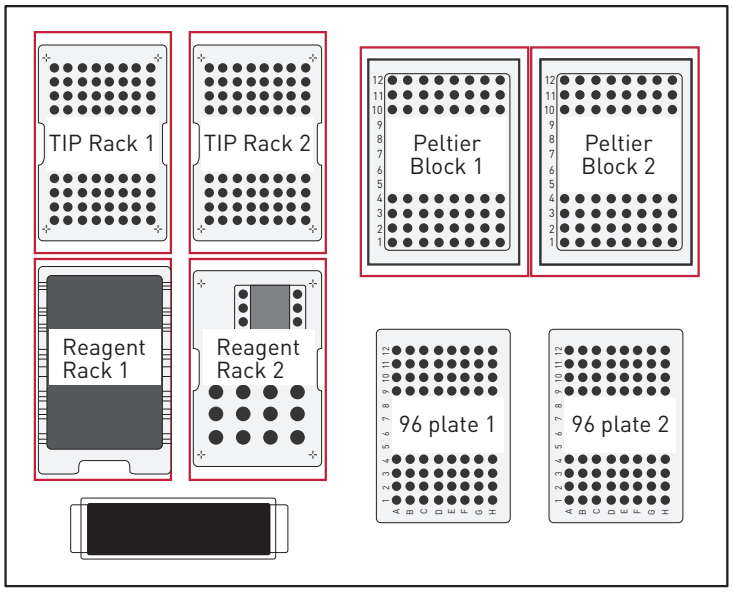
3.5. Setup the parameters for your ChIPmentation experiment and press "Next"

Recommended parameters:



NOTE: The recommended tagmentation time is 10 minutes, but the optimal time can vary depending on the cell number and the antibody used. See "Remarks before starting" section for more detail

3.6. Setup all the plastics on the platform according to the screen layout.



- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Reagent Racks 1 & 2** with reagent containers according to the screen.
- Fill **Peltier Block 1** (and 2 if processing more than 8 samples) with 8-tube strips according to the screen.

NOTE: All the rows of the Peltier(s) Block(s) must be filled with a strip.

3.7. Fill the strips with your samples and the reagents from the kit as described below and make sure that the liquid is at the bottom of each well.

- Distribute **20 µl of DiaMag Protein A-coated magnetic beads** in each well of row 3.
- Prepare **ChIP Buffer** as described in the table below. The volumes are in µl and contain an excess.

	1 IP	2 IPs	3 IPs	4 IPs	5 IPs	6 IPs	7 IPs	8 IPs
5x ChIP Buffer iC1	80	240	320	400	480	560	640	720
ChIP-seq grade Water	320	960	1280	1600	1920	2240	2560	2880
TOTAL ChIP Buffer	400	1200	1600	2000	2400	2800	3200	3600

	9 IPs	10 IPs	11 IPs	12 IPs	13 IPs	14 IPs	15 IPs	16 IPs
5x ChIP Buffer iC1	960	1040	1120	1200	1280	1360	1440	1520
ChIP-seq grade Water	3840	4160	4480	4800	5120	5440	5760	6080
TOTAL ChIP Buffer	4800	5200	5600	6000	6400	6800	7200	7600

- Prepare the **Ab coating mix** as described in the table below and distribute 100 µl in each well of row 6.

Antibody	x µl
ChIP Buffer	100 – x µl
200x Protease Inhibitor cocktail	0.5 µl
5% BSA (DNA free)	2 µl

NOTE: The required amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody. Use 1 µg of IgG (negative control antibody) for the negative control IP. If a positive control IP is included, use 1 µg of the H3K4me3 positive control antibody.

- Prepare the **Immunoprecipitation mix** as described in the table below and distribute 200 µl in each well of row 7.

Sheared chromatin	100 µl
ChIP Buffer	100 µl
BSA 5%	4 µl
200x Protease Inhibitor Cocktail	1 µl

- Keep aside **1 µl of the sheared chromatin** at 4°C to be used as an **INPUT** starting from step 4 point 4.4.

NOTE: If required, NaBu (HDAC inhibitor, 20 mM final concentration) or other inhibitors can also be added to the chromatin sample.

- Distribute **1 µl of Tagmentation enzyme** in each well of row 2.

3.8. Fill **Reagent Racks 1 & 2** with reagents according to the screen instructions.

3.9. Check the proper insertion of the racks and the consumables, and press "Next".

3.10. Check the selected parameters, close the door, and press "Run" to start.

3.11. ChIPmentation is running. The "Remaining time" calculation will give you an estimation of the processing time of your experiment.

STEP 4

Stripping, end repair, reverse cross-linking

1-1.5 hour

4.1. The next morning, after the overnight incubation, recover the sample tubes in row 12. The volume in each tube is 150 µl. Press "OK" and "Back" until the homepage appears on the screen.

4.2. Briefly spin the strip and place it on DiaMag02 magnetic rack (Cat. No. B04000001).

4.3. Wait until supernatant is clear and discard the supernatant.

4.4. Remove the strip from magnetic rack, add **10.5 µl of Stripping Reagent** to the beads and resuspend by pipetting. Add **9.5 µl of Stripping Reagent** to the INPUT.

4.5. Heat the immunoprecipitated and input samples 30 minutes at 50°C using a thermocycler.

NOTE: During incubation time remove all the plastics from the IP-Star® platform, empty the waste shuttle, and clean the inner side of the IP-Star® with 70% ethanol.

4.6. Preheat the needed amount of **PCR MasterMix** 30 seconds at 98°C on a heating block. **25 µl of PCR MasterMix** will be needed for each IP'ed or input sample.

4.7. Add **10.5 µl $MgCl_2$** and **25 µl of preheated PCR MasterMix** to each IP'ed and input samples and incubate as follows:

Temperature	Time
72°C	5 minutes
95 °C	10 minutes
Cooling at 4 °C (or ice)	

4.8. Magnetize beads from the immunoprecipitated samples and transfer the supernatant to a new IP-Star strip (Cat. No. C30020002).

STEP 5

Library amplification

2.5 hour

Optional: Take 3 µl of each immunoprecipitated and input sample, dilute it 8x with water and use it to perform a qPCR to check the % of recovery of one positive and one negative control region. The protocol for qPCR analysis is described in a separate section "Protocol for quantitative PCR analysis".

Determination of the optimal cycle number for the enrichment PCR

NOTE: for this step only 2 µl of each library will be used.

5.1. Preheat the needed amount of PCR Master Mix 30 seconds at 98°C. **5 µl of PCR MasterMix** will be needed for each IP'ed sample.

NOTE: Starting from this step the input samples are not processed anymore.

5.2. Prepare the **Quantification Mix** as described in the table below for the number of desired reactions. Mix by pipetting and keep on ice until use.

Component	Volume per reaction
Universal Primer	0.3 µl
Primer Index 1	0.3 µl
Preheated PCR MasterMix	5 µl
100x SYBR	0.1 µl
Nuclease-free Water	2.3 µl

5.3. Dispense **8 µl of the Quantification Mix** into 0.2 ml tubes or strips according to the number of libraries.

5.4. Add **2 µl of IP'ed DNA** to each tube and mix by pipetting.

5.5. Briefly spin the tubes and run the PCR program described below.

Cycles	Temperature	Time
1	98°C	30 seconds
25	98°C	10 seconds
	63°C	30 seconds
	72°C	30 seconds
1	72°C	1 minute
1	10°C	∞

5.6. Analyse the Ct values. The optimal cycle number for the amplification of the rest of the ChIPmentation DNA is typically Ct (rounded up) +2.

NOTE: The Ct value is highly dependent on the thermocycler you use, as well as the way you analyze the qPCR results. Thus when using the kit for the first time you may need to verify that the Ct+2 rule applies well in your conditions.

5.7. Add **1.5 µl of Universal Primer** to each tube from step 4.8.

NOTE: The tubes already contain the mastermix as it was added at step 4.6.

5.8. Add **1.5 µl of the appropriate Primer Index** in each tube and mix by pipetting.

NOTE: Use different indexes for samples that you want to sequence in the same lane (see index sequences and pooling recommendations in the Kit materials section page 9).

5.9. Briefly spin the tubes and run the PCR program described below.

Cycles	Temperature	Time
1	98°C	30 seconds
X (Ct rounded up +2)	98°C	10 seconds
	63°C	30 seconds
	72°C	30 seconds
1	72°C	1 minute
1	10°C	∞

NOTE: After amplification it is possible to use 1µl of library to run on a 2100 BioAnalyzer (Agilent) when keeping the samples on ice. It permits to check that enough material was generated. If needed additional amplification cycles can then be performed. However higher yields may come at the expense of reduced sequencing quality. Therefore we recommend not using more than 18 cycles in order to avoid an over-amplification.

STEP 6

Clean-up

20 minutes hands on time
40 min run for each series of 8 samples

Use the IP-Star® and room temperature AMPure XP beads for the clean-up.

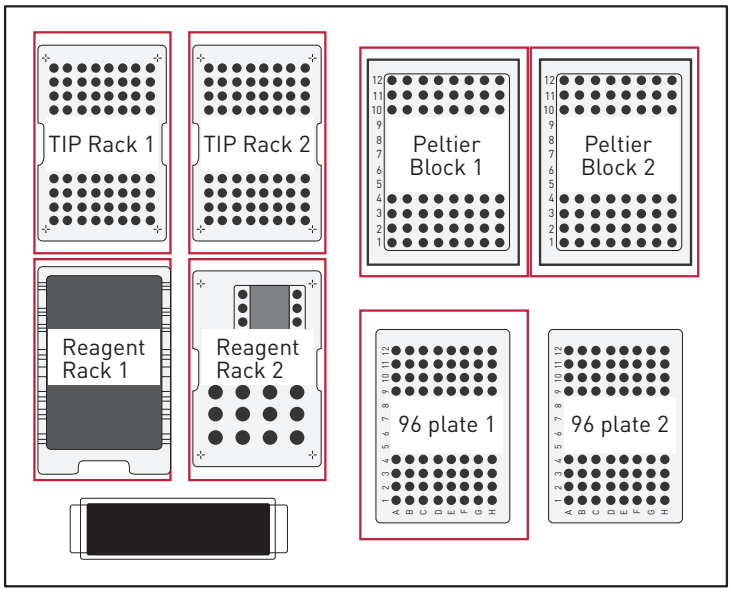
6.1. Select "**Protocols**" icon and then "**ChIPmentation**" category.

6.2. Select "**AMPure_XP_Purification_08**" if you plan to run between 1 and 8 samples, or "**AMPure_XP_Purification_16**" if you plan to run between 9 and 16 samples.

6.3. Setup the exact number of samples that you want to process by pressing the black box.

NOTE: The Left Peltier Block is now cooling down to 4°C to keep your samples cold.

6.4. Setup all the plastics on the platform according to the screen layout.



- Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
- Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
- Fill **96 plate 1** with a 96 well microplate.
- Fill **Left Peltier Block** with 200 µl tube strips according to the screen

6.5. Fill the robot with all reagents.

- Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Left Peltier Block**.
- Distribute room temperature AMPure XP Beads in row 1 (and 5 if processing more than 8 samples) of the **96-well Microplate**. The amount of beads must be 1.8x the volume of sample (e.g. for 45 µl of samples use 81 µl of beads).

NOTE: Resuspend the beads with pipetting up and down several times before dispense them.

- Fill the container of the **Reagent Rack 1** with freshly prepared 80% Ethanol according to the screen.
- Fill the container of **Reagent Rack 2** with Resuspension Buffer according to the screen.
- Check the proper insertion of the racks and the consumables.

6.6. Close the door and press "Run" to start.

6.7. After the run, recover your samples on the upper row of the **Left Peltier Block**. The final volume is 20 µl per each sample.

6.8. Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star®.

NOTE: Remove all the plastics from the IP-Star platform, empty the waste shuttle, and clean the inner side of the IP-Star® with 70% ethanol.

STEP 7

Quality control

7.1. Determine the concentrations of your samples by the use of a fluorescence-based assay such as the Qubit High Sensitivity assay (ThermoFischer Scientific).

7.2. Run a part of each library on a High Sensitivity chip for BioAnalyzer (Agilent) or on Fragment Analyzer (Advanced Analytical) according to the manufacturer's instructions.

NOTE: In some cases, for example when large fragments are still present, a size selection can be performed (see the "Additional protocols" section, page 45).

7.3. Your libraries are now ready for pooling and sequencing.

NOTE: Individual libraries, quantified and purified according to the above protocol, can be pooled at desired molar ratios to allow multiplex sequencing. Libraries that are being pooled must have been prepared with different indexes (for multiplexing and index pooling guidelines refer to Indexes, page 9). The minimal molar concentration needed to sequence the pool depends on the requirements of the sequencing platform. The total molarity is the sum of all the individual libraries' molarities in the final volume, e.g. if you add 5 µl of a 10 nM library to 5 µl of a 20 nM library, you have 10 µl of a 15 nM pool. If libraries are prepared from similar input amounts, they can be pooled by combining equal volume aliquots of each library.

?

ASK THE EXPERTS

Please, do not hesitate to contact our customer support team if you have any questions about the design of your ChIPmentation experiment or the bioinformatics data analysis.

Contact for Europe, Asia, Oceania and Africa:
custsupport@diagenode.com
Contact for North and South America:
custsupport.na@diagenode.com

ChIP-seq data analysis recommendations

ChIP-seq data analysis workflow

In the following chapter we will guide you through the basics of ChIP-seq data analysis. We will also provide some examples of software tools suitable for each step. We cite numerous analysis tools, including free and commercial softwares.

1. (Optional step) Trimming: use trimming to get rid of low quality bases and artefacts in the readset, such as adapter contaminations
    a. Cutadapt
    b. Trim Galore!
    c. Trimmomatic
2. Alignment: in this step you will map the reads against a known reference sequence
    a. ELAND
    b. Tmap
    c. BWA
    d. Bowtie2
3. (Optional step) Quality control: you can check the general quality of the sequencing and the alignment
    a. FastQC
    b. Picard Tools
4. Peak calling: during peak calling the software will detect sites of enrichment along the genome
    a. MACS2
    b. SICER
    c. ZINBA
    d. PeakRanger

e. Pyicoteo
f. MUSIC
g. SPP
h. hiddenDomains

After above described basic analysis, the peaks can be analyzed further to get answers to our biological questions. There are countless ways and tools for further analyses, the project goals determine which ones we should pick. Just as in the case of basic analysis, we recommend to thoroughly study the manual of the chosen software tool to understand its purpose and its function. Below we will list a few common analysis types to further process the peaks, along with example tools again.

5. Visualization: the peaks, the reads, and other data (e.g. gene positions) can be displayed in a suitable genome browser
   a. IGV
   b. IGB
   c. USCS Genome Browser
6. Descriptive statistics: the peaks can be described in various useful ways, like how many reads fall into them, what is their number, mean size and significance etc.; these figures are also very useful when you compare datasets
   a. Peak callers usually provide per peak and/or summary statistics after peak detection
   b. HOMER
   c. GREAT
   d. BEDTools
7. Motif search: in the case of TF data peaks frequently occur at specific motifs, though some HM peaks can also show preference to certain sequence patterns; therefore identifying these motifs and checking enrichments over them is a good practice for TF data analysis, and also applicable for HM data.
   a. HOMER
   b. MEME Suit

8. Annotation, Gene Ontology, Pathway analysis: after annotation/GO/Pathway analysis you will get a clear picture about which genomic features or pathways your peaks are associated to, providing an important information about disease mechanisms, the role of DNA binding proteins, or treatment effects

   a. HOMER
   b. GREAT
   c. BEDTools
   d. ReactomePA

9. Comparative analysis: this type of analysis is the obvious choice when you have several datasets from comparable conditions (e.g. treated and untreated cells) or when you want to check the performance of your ChIP-seq by comparing it to a reference; there are many different ways to compare peaks, including checking the overlaps, the correlation of enrichment sizes and performing statistical tests on the peaksets

   a. HOMER
   b. BEDTools
   c. DiffBind

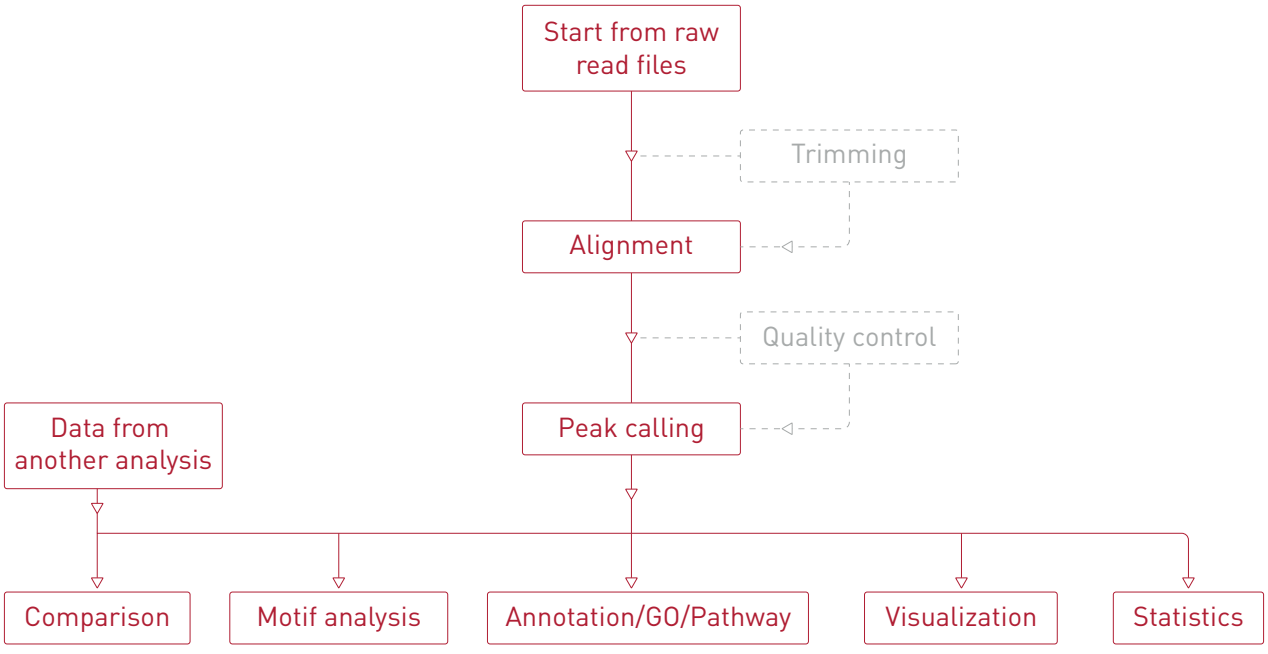


Figure 1. ChIP-seq data analysis workflow

Example of results

Recovery analysis (after Step 4)

ChIPmentation was performed on human K562 cells in duplicates. Sheared chromatin from 1 million cells was used per IP, in combination with 1 µl of the positive control antibody H3K4me3 (Cat. no. C15410003) or 2 µl of the negative IgG control. Ten minutes tagmentation were applied. The IP efficiency was checked by qPCR.

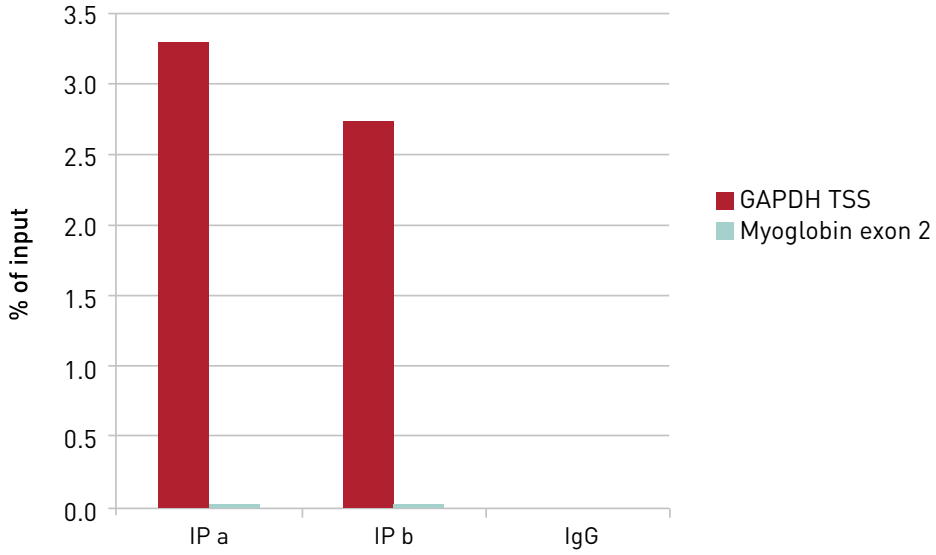


Figure 2: Recovery analysis. Quantitative PCR was performed with the positive control GAPDH-TSS and the negative control Myoglobin exon 2 primer sets from the kit. The recovery is expressed as a % of input and is the relative amount of immunoprecipitated DNA compared to input DNA.

Quality control of the libraries before sequencing (Step 7)

After amplification and purification, the H3K4me3 ChIPmentation libraries were analyzed on BioAnalyzer (Agilent).

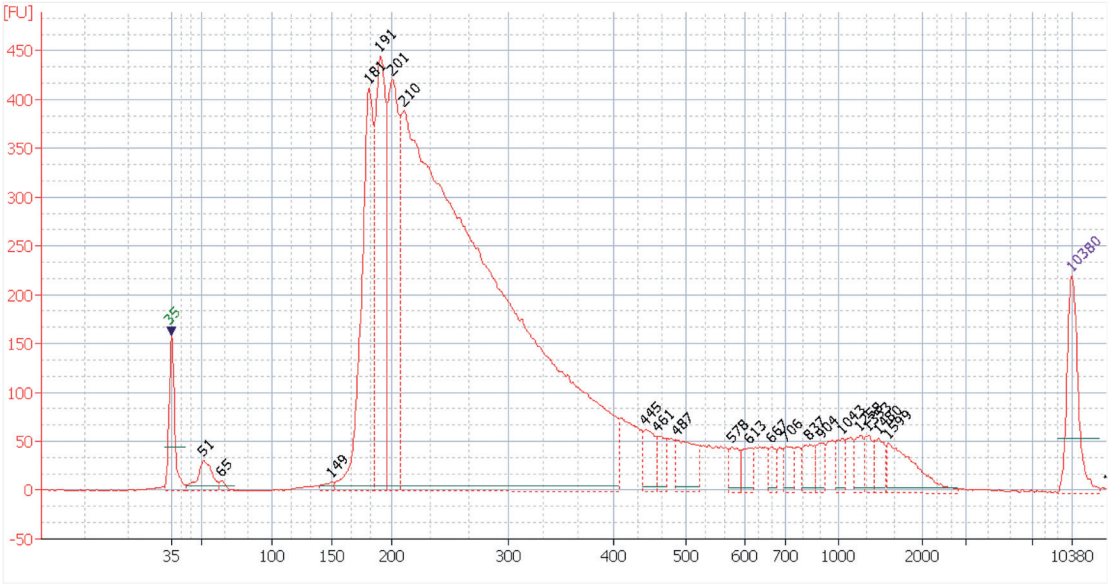


Figure 3: Library quality control. The distribution of the fragments sizes was assessed by loading 6 ng of library on BioAnalyzer (Agilent).

Sequencing

ChIPmentation libraries were finally sequenced on Illumina's HiSeq3000/4000 and the data analyzed as described page 34.

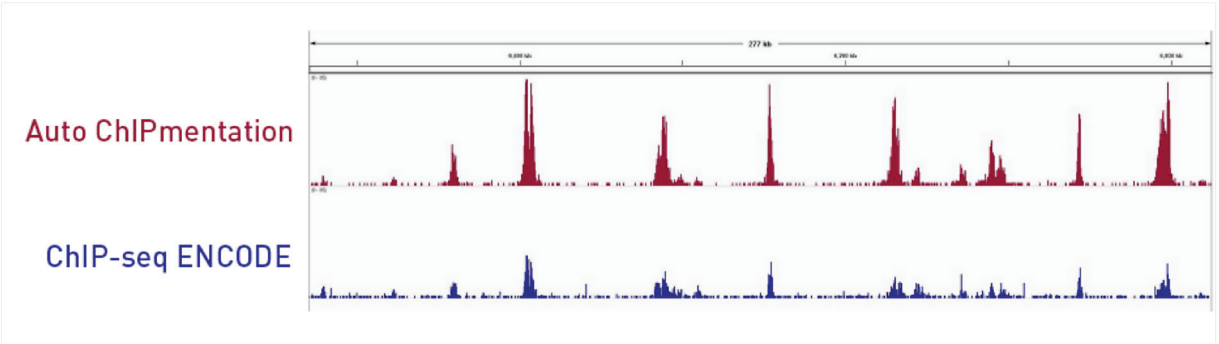


Figure 4. Distribution of the ChIPmentation and ENCODE datasets for H3K4me3 ChIP-seq, in a representative region of the genome.

ADDITIONAL PROTOCOLS

Protocol for chromatin shearing analysis

General remarks

We recommend using an agarose gel analysis or the Fragment Analyzer (Advanced Analytical) for the size assessment.

For accurate size determination of the chromatin fragments, reverse crosslinking, including RNase treatment followed by DNA purification, is advised. Size estimation of chromatin fragments without reverse cross-linking is not precise. The presence of the crosslinks retards electrophoretic migration resulting in a misinterpretation of fragment size. RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing.

Workflow for analysis of sheared chromatin:

- RNAse treatment (1h, optional but highly recommended for an accurate size assessment)
- Reverse crosslinking (4h or overnight)
- DNA purification using IPure beads (v.2) (30 minutes)
- Fragment size assessment (agarose gel or Fragment Analyzer) (1h)

RNAse treatment

NOTE: RNase cocktail (e.g. Ambion, AM2286A) is not supplied with this kit.

1. Take **50 µl of sheared chromatin** (end of step 2 in the protocol) and transfer to a 1.5 ml microtube.

2. Dilute **1 µl of RNase cocktail** (e.g. Ambion, AM2286A) in **150 µl of ChIP-seq grade water**.

3. Add **2 µl of diluted RNase cocktail** to the aliquot of sheared chromatin.

4. Incubate for 1 hour at 37°C.

Reverse cross-linking

5. Add **50 µl of Elution buffer iE1**.

6. Add **4 µl of Elution buffer iE2**, mix thoroughly.

7. Incubate samples at 65°C for 4 hours (or overnight).

DNA purification

NOTE: The protocol below describes DNA purification using the IPure magnetic beads included in the kit. Other methods of DNA purification (columns-based DNA clean-up, e.g. DiaPure columns from Diagenode or a phenol–chloroform extraction followed by ethanol precipitation) can be used also.

NOTE: Before the first use of the kit, prepare Wash buffer 1 and Wash buffer 2 by adding an equal volume of isopropanol. Wash buffers 1 and 2 should be stored at 4°C. Never leave the bottle open during storage to avoid evaporation.

8. Add **2 µl of carrier** to the sample.

9. Add **108 µl of 100% isopropanol** to the samples.

NOTE: Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.

10. Resuspend the **IPure beads v2** by vortexing and add **20 µl** to the sample.

11. Incubate samples for 10 minutes at room temperature on the DiaMag Rotator.

12. Briefly spin the tubes, place in the DiaMag1.5 magnetic rack, wait 1 minute and discard the buffer.

13. Add **100 µl of Wash buffer 1** per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for 30 seconds at room temperature. Briefly spin the tubes, place in the DiaMag1.5 magnetic rack, wait 1 minute and discard the buffer.

14. Add **100 µl of Wash buffer 2** per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for

30 seconds at room temperature. Briefly spin the tubes and place them into the DiaMag 1.5 magnetic rack, wait 1 minute and discard the buffer.

15. Spin the tubes again and place them on the DiaMag1.5. Discard the remaining Wash buffer 2 if necessary. Resuspend the beads pellet in **25 µl of buffer C**. Incubate at room temperature for 15 minutes on the DiaMag Rotator.

16. Spin the tubes and place them into the DiaMag 1.5, wait 1 minute and transfer the supernatants into a new 1.5 ml tube. Discard the beads.

Fragment size assessment

Analyze the purified DNA on a 1.5% agarose gel. Load around 300 ng of DNA for an optimal separation and visualization. Alternatively, you can use a Fragment Analyzer (Standard Sensitivity NGS Fragment Analysis Kit (DNF-473)).

Protocol for quantitative PCR analysis

NOTE: For each primer pair, run the Input DNA alongside the immunoprecipitated samples and negative IgG control.

1. Prepare the qPCR mix as follows (20 µl reaction volume using the provided control primer pairs):
   - 10 µl of a 2x SYBR® Green qPCR master mix
   - 1 µl of primer pair
   - 4 µl of water
   - 5 µl of diluted IP'ed or INPUT DNA

2. Use the following PCR program:

   NOTE: These conditions may require optimization depending on the type of Master Mix, qPCR system used and user provided primer pair.

Step	Time/cycles		Temperature
1. Denaturation	3-10 min*		95°C
2. Amplification	30 seconds	40 cycles	95°C
	30 seconds		60°C
	30 seconds		72°C (acquire fluorescence data)
3. Melting curve**	Follow qPCR instrument manufacturer recommendations		

   ** Please check carefully supplier's recommendations about Taq polymerase activation time*

   *** Include and inspect the melting curves based on the protocols recommended by the qPCR instrument manufacturer to ensure that primer pairs amplify only a single specific product*

3. Record the threshold cycles (Ct values) from the exponential phase of the qPCR for the IP'd DNA sample and input for each primer pair.

4. Calculate the relative amount of immunoprecipitated DNA compared to INPUT DNA for the control regions (% of recovery) using the following formula:

% recovery = $2^{(Ct_{input} - Ct_{sample})}$

- Ct_{sample} and Ct_{input} are the threshold cycles from the exponential phase of the qPCR for the IP'd DNA sample and INPUT, respectively.
- 2 is the amplification efficiency

NOTE: This equation assumes that the PCR is 100% efficient (amplification efficiency = 2). For accurate results, the amplification efficiency with given primer pair has to be close to 100% meaning that for each cycle the amount of product is doubles (E=2). The real amplification efficiency, if known, should be used.

The formula takes into account that 1% of input was used as suggested in the protocol (2 µl INPUT vs 200 µl of chromatin per IP). If the amount of INPUT used is different from 1%, an introduction of a compensatory factor in the formula is required to correct the input dilution (x) as follows:

%recovery = $2^{[(Ct_{input}-log2(X\%)\ Ct_{sample})]}$x100%

Where: log2(x) accounts for the INPUT dilution

Example: if you use an INPUT of 5 µl from 250 µl of chromatin used per IP, it corresponds to 50 X dilution. The compensatory factor is equal to log2(50)=5.64 and the formula to calculate the recovery will be as follows:

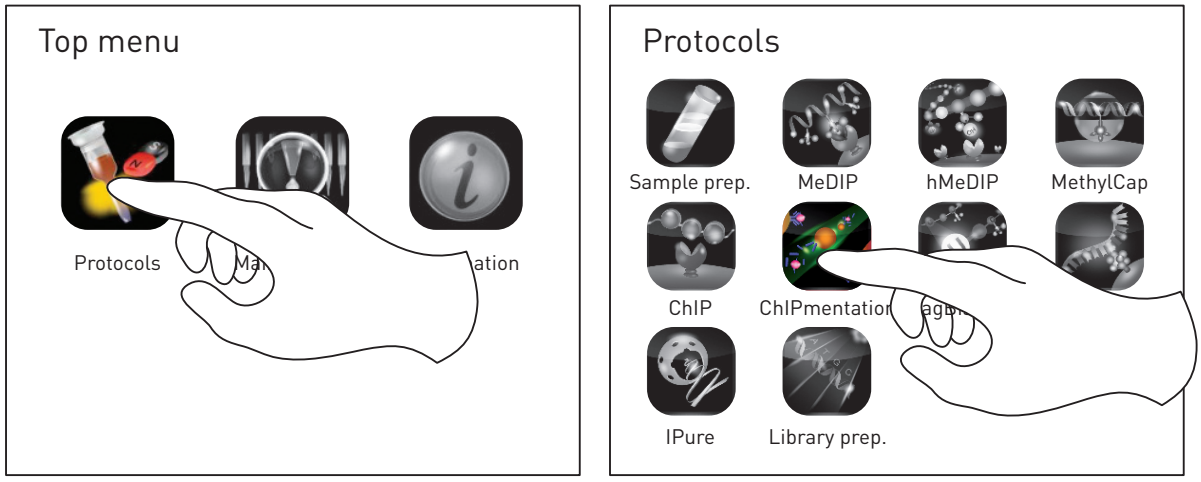
%recovery = $2^{[(Ct_{input}-5.64\ Ct_{sample}]}$ x100%

Protocol for size selection on the IP-Star® Compact

30 minutes hands on time
1h10 minutes run for each series of 8 samples

Use the IP-Star® and room temperature AMPure XP beads for the size selection.

1. Switch on the IP-Star® and select "**Protocols**" icon and then "**ChIPmentation**" category.



2. Select "**ChIPmentation_Size_Selection_08**" if you plan to run between 1 and 8 samples, or "**ChIPmentation_Size_Selection_16**" if you plan to run between 9 and 16 samples.

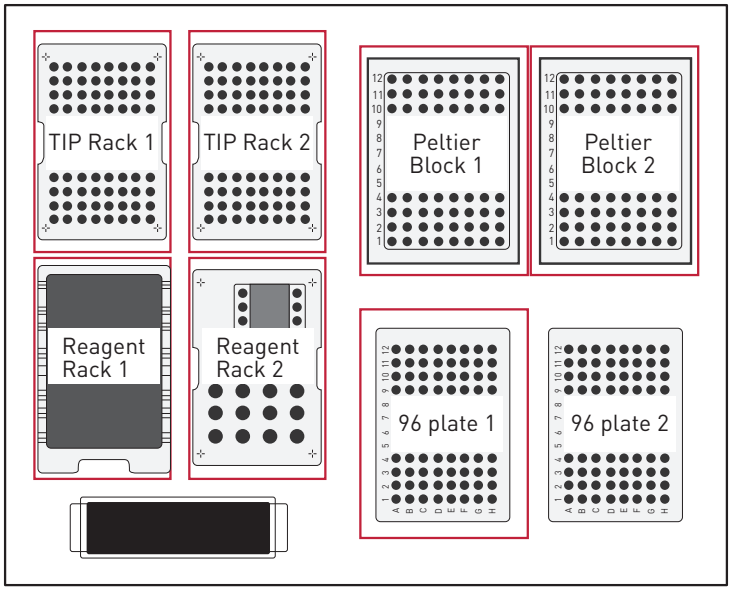
3. Setup the exact number of samples that you want to process by pressing the black box.

   NOTE: The Left Peltier Block is now cooling down to 4°C to keep your samples cold.

4. Setup all the plastics on the platform according to the screen layout.

   - Fill **TIP Rack 1** (and 2 if processing more than 8 samples) with tips according to the screen.
   - Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.

- Fill **96 plate 1** with a 96 well microplate.
- Fill **Left Peltier Block** with 200 µl tube strips according to the screen.



5. Fill the robot with all reagents.

   - Add 80 µl of nuclease-free water to each sample to have a final volume of 100 µl. Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Left Peltier Block**.
   - Distribute 90 µl of room temperature AMPure XP Beads in row 1 (and 7 if processing more than 8 samples) of the 96-well Microplate.

   NOTE: Resuspend the beads with pipetting up and down several times before dispense them.

   - Fill the container of the **Reagent Rack 1** with freshly prepared 80% Ethanol according to the screen.
   - Fill the container of **Reagent Rack 2** with Resuspension Buffer according to the screen.
   - Check the proper insertion of the racks and the consumables

6. Close the door and press "Run" to start.

7. After the run, recover your samples on the upper row of the Left Peltier Block. The final volume is 20 µl for each sample.

8. Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star®.

NOTE: Remove all the plastics from the platform, empty the waste shuttle and clean the inner side of the IP-Star® with 70% ethanol.

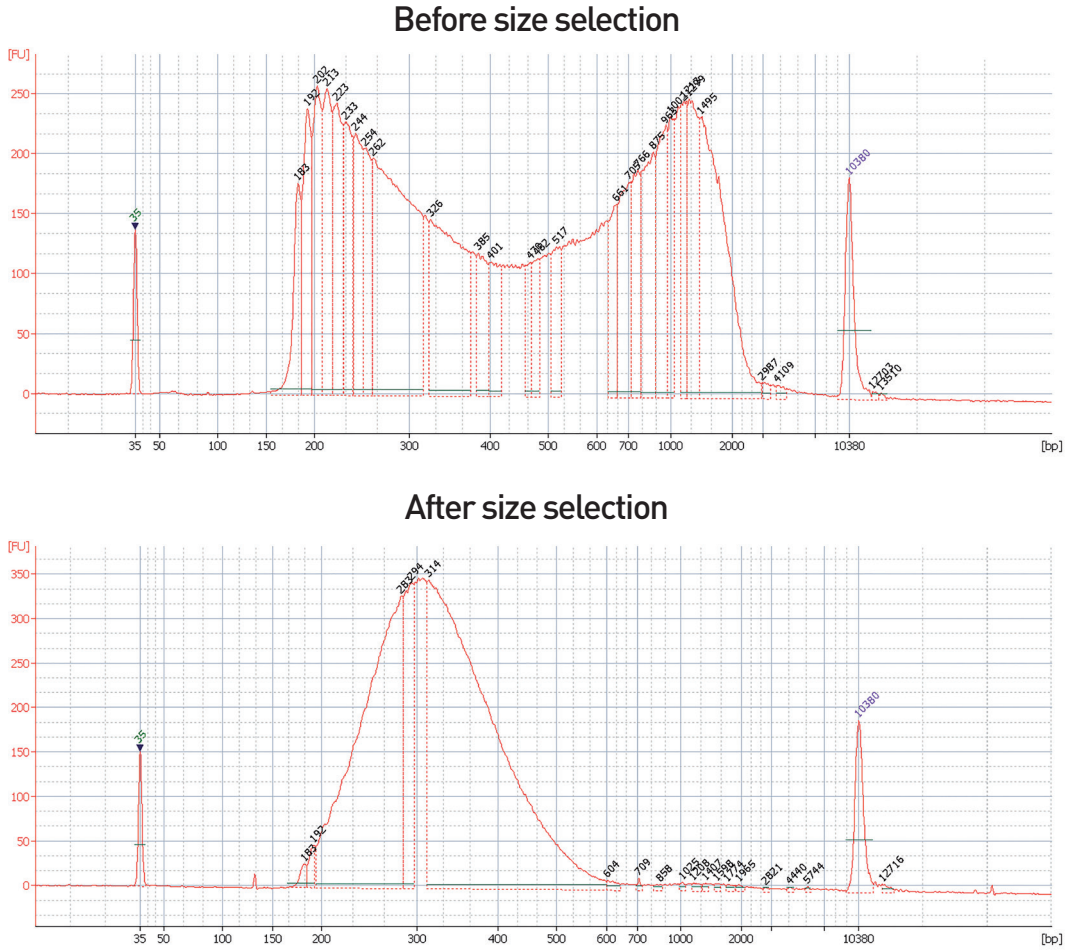


Figure 5. Example of results of the size selection on a library for H3K27me3 ChIP-seq. The library was run on a BioAnalyzer (Agilent) before and after the size selection.

FAQs

Is the included control H3K4me3 antibody compatible with mouse?

Yes, the included control H3K4me3 antibody is compatible with mouse.

How much antibody is needed per IP?

The amount of antibody needed depends on different factors of which the antibody itself is the most important. Most of Diagenode ChIP-seq grade antibodies have been tested at different concentrations to determine the optimal amount and the suggested amount of antibody is given in the data sheet. Please note, however, that this can be assay dependent and might need to be optimized for each experimental setting. If the antibodies come from other companies, please refer to the corresponding technical data sheet. If no required amount of antibody is given, we suggest performing a titration experiment. For ChIP-seq it is important to select the amount of antibody which gives the lowest background signal.

What is the binding capacity of DiaMag Protein A-coated magnetic beads?

30 µl of DiaMag Protein A-coated magnetic beads can bind 10 µg of antibody.

What is the specificity of protein A-coated magnetic beads?

Ideal ChIP kit for histones contains DiaMag Protein A-coated magnetic beads which allow an efficient capture of rabbit, guinea pig, dog and pig polyclonal and monoclonal Abs, mouse IgG2a, IgG2b and IgA and human IgG1, IgG2 and IgG4. If the antibody of interests belongs to a different class of immunoglobulins (mouse IgG1 and IgG3, rat or goat polyclonal Abs, and human IgG3), DiaMag Protein G-coated magnetic beads should be used instead of protein A coated beads. These beads are available separately (C03010021-220).

Can I use a monoclonal antibody in ChIP experiment?

ChIP can be performed using either monoclonal or polyclonal antibodies.

In general, polyclonal antibody populations will recognize a number of different epitopes, in contrast to monoclonal antibodies, which recognize a single epitope. Because monoclonals recognize a single epitope on a target protein, they often provide a high level of specificity, low non-specific binding, and low background signals. The major disadvantage of a monoclonal antibody is its recognition of only one epitope, which can be masked by cross-linking, decreasing the efficiency of immunoprecipitation.

What is the composition of buffers included in the kit?

The composition of the buffers is proprietary.

What are the expected concentration and size of ChIPmentation libraries?

The concentration of libraries that you need to reach will depend on the sensitivity of the machine and kits that you will use to perform the quality control and the sequencing of your libraries. Usually a concentration of 4-8 ng/µl is enough for a quality control using the Qubit High Sensitivity assay (ThermoFischer Scientific) and the High Sensitivity chip for BioAnalyzer (Agilent) and for sequencing on Illumina HiSeq3000/4000.

Regarding the size, ideally the library should show fragments around 200-500 bp. If some larger fragments are present the best would be to contact your sequencing provider to ask what are their requirements, because it can vary depending on the sequencer. If you want to remove the large fragments you can use the size selection protocol described in the manual.

Regarding the questions related to the **IP-Star® Compact**, please refer to the troubleshooting guide and the list of error codes pages 29-31 of the SX-8G IP-Star® Compact manual.

Related products

Product	Cat. No.
Chromatin shearing optimization kit - Low SDS (iDeal Kit for Histones)	C01020010
DiaMag Rotator	B05000001
DiaMag0.2	B04000001
Bioruptor Pico®	B01060001
IP-Star® Compact Automated System	B03000002

Validated antibodies – check out the complete list at www.diagenode.com

ChIP-seq grade antibody	Cat. No.
H2A.Z polyclonal antibody	C15410201
H2BK15ac polyclonal antibody	C15410220
H3K27ac polyclonal antibody	C15410196
H3K27me3 polyclonal antibody	C15410195
H3K36me3 polyclonal antibody	C15410192
H3K4me1 polyclonal antibody	C15410194
H3K4me3 polyclonal antibody	C15410003
H3K79me3 polyclonal antibody	C15410068
H3K9/14ac polyclonal antibody	C15410200
H3R17me2(asym)K18ac polyclonal antibody	C15410171
H4K20ac monoclonal antibody	C15210008
H4K20me3 polyclonal antibody	C15410207
macroH2A.1/H2A.2 monoclonal antibody	C15210003

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