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Innovating Epigenetics Solutions

True MicroChIP-seq Kit

Cat. No. C01010132

USER GUIDE

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Please read this manual carefully
before starting your experiment

Summary

Introduction

Association between proteins and DNA has a major influence on gene transcription, epigenetic silencing and other nuclear processes which control many vital cellular functions. It is crucial to understand these interactions and the mechanisms by which they control and guide gene regulation pathways and cellular proliferation. **Chromatin immunoprecipitation (ChIP)** is a technique to analyze the association of proteins with specific genomic regions in intact cells. ChIP can be used to study changes in epigenetic signatures, chromatin remodeling and transcription regulator recruitment to specific genomic sites.

Traditional ChIP protocols require large numbers of cells, limiting the application for ChIP technology to few cell samples. The **True MicroChIP-seq kit** provides a robust ChIP protocol suitable for the investigation of histone modifications within chromatin from cells as few as **10 000 cells**, including **FACS sorted cells**.

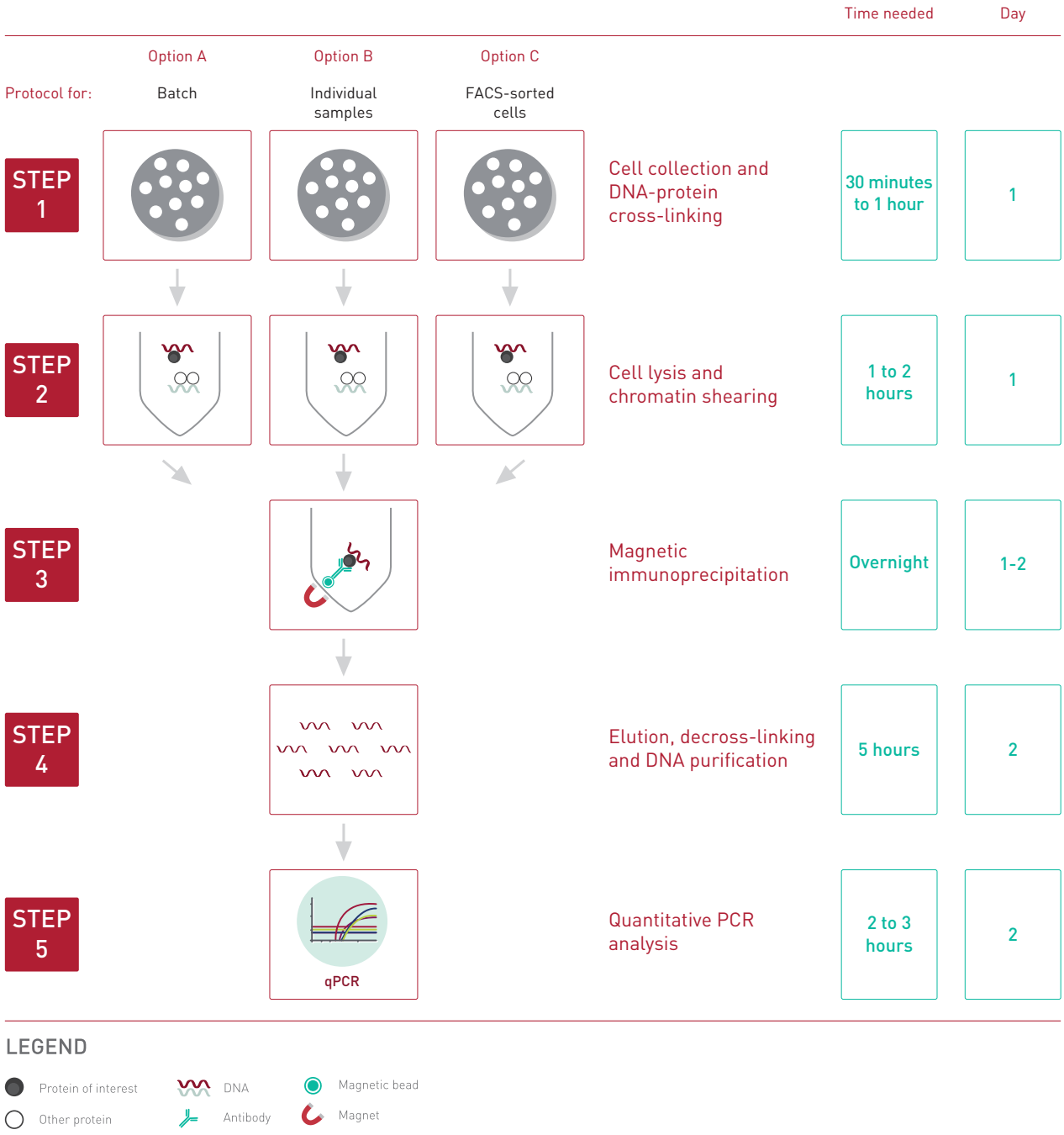
The protocol involves protein-DNA cross-linking with formaldehyde, followed by cell lysis and fragmentation of the cross-linked chromatin. The subsequent immunoprecipitation of chromatin is performed with an antibody (user-supplied) specific to a target histone modification. Protein A magnetic beads are used to isolate the protein-DNA complexes of interest. The immunoprecipitated DNA is eluted and purified using DiaPure columns. The eluted DNA can be used for **qPCR** and/or for **ChIP-seq library preparation**. Additionally, the kit includes a negative (IgG) and a positive (H3K4me3) control antibody and primer pairs amplifying H3K4me3 positive and negative loci.

The True MicroChIP-seq kit offers unique benefits:

- An optimized chromatin preparation protocol compatible with low number of cells in combination with the Bioruptor™ shearing device
- Most complete kit available (covers all steps and includes control antibodies and primers)
- Magnetic beads make ChIP easy, fast, and more reproducible
- Easy-to-follow protocol
- DiaPure columns enable the maximum recovery of immunoprecipitation DNA suitable for any downstream application
- Excellent ChIP-seq result when combined with MicroPlex Library Preparation kit adapted for low input

Kit Method Overview & Time Table

The True MicroChIP-seq kit protocol has been optimized for the use of **10,000-100,000 cells** per immunoprecipitation reaction. Regarding chromatin immunoprecipitation, three protocol variants have been optimized: starting with a **batch**, starting with an **individual sample** and starting with the **FACS-sorted cells**. Please refer to the corresponding protocol section.



Kit Materials

The True MicroChIP-seq Kit contains all reagents necessary for chromatin preparation, immunoprecipitation and DNA purification.

Table 1. Number of reactions included in the True MicroChIP-seq Kit

Number of			
Chromatin preparation*	ChIP reaction	Input	Shearing controls
20	20	20	20

**From individual samples, FACS-sorted or batch up to 10 samples.*

Table 2. Components supplied with the True MicroChIP-seq Kit

Description	Quantity	Storage
Glycine	4.5 ml	4°C
Lysis Buffer tL1	1 ml	4°C
Protease inhibitor cocktail 200x (PIC)	225 µl	20°C
ChIP Buffer tC1	4.5 ml	4°C
Beads Wash Buffer tBW1	13.6 ml	4°C
Protein-A coated magnetic beads	200 µl	4°C (Do NOT Freeze!)
Wash Buffer tW1	4.5 ml	4°C
Wash Buffer tW2	4.5 ml	4°C
Wash Buffer tW3	4.5 ml	4°C
Wash Buffer tW4	4.5 ml	4°C
Elution Buffer tE1	11 ml	4°C
Elution Buffer tE2	410 µl	4°C
Control IgG (rabbit) µg	8 µg	4°C
ChIP-seq grade antibody H3K4me3	8 µg	-20°C
Human GAPDH TSS primer pair	40 µl	-20°C
Human Myoglobin exon 2 primer pair	40 µl	-20°C
ChIP DNA Binding buffer	50 ml	RT
DNA Wash buffer*	6 ml	RT
DNA elution buffer	10 ml	RT
Spin columns	50	RT
Collection tubes	50	RT

***NOTE**: Upon receipt, store the components at the indicated temperature*

**Ethanol must be added as indicated on the label.*

Required Materials Not Provided

Materials and Reagents

- Gloves to wear at all steps
- RNase/DNase-free 1.5 ml tubes
- RNase/DNase-free 15 ml and 50 ml tubes
- RNase/DNase-free 0.2 ml tubes (or 8-tube strips)
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS) buffer
- 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
- Cell culture medium
- Trypsin-EDTA
- Hank's balanced salt solution (HBSS – e.g. ThermoFisher Scientific, 14175095)
- RNase cocktail (e.g. Ambion, AM2286) (for chromatin shearing assessment)
- Supplies for FACS-sorted cells
- (Optional) LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation or similar (Thermo Fischer Scientific)
- Cell strainer (<80µm)
- 5% BSA (Diagenode, Cat. No. C03020005)

Equipment

- Cell counter system
- Fume hood

- Bioruptor® sonication device and the associated microtubes:
  - Bioruptor Pico (Diagenode, Cat. No. B01060010),
    - 0.65ml microtubes (Cat. No. C30010011) or
    - 1.5 ml microtubes with caps (Cat. No. C30010016)
  - Bioruptor Plus (Diagenode, Cat. No. B01020003),
    - 0.5ml microtubes (Cat. No. C30010013) or
    - 1.5 ml TPX microtubes (Cat. No. C30010010-300)
- Refrigerated centrifuge for 1.5 ml and 0.2ml tubes
- Centrifuge for 15 ml and 50 ml tubes
- DiaMag Rotator (Rotating wheel) (Diagenode, Cat. No. B05000001)
- Magnetic rack for 1.5 ml tubes: DiaMag1.5 (Diagenode, Cat. No. B04000003)
- qPCR cycler
- Thermocycler
- qPCR SYBR® Green Mastermix, for quantitative PCR analysis
- Sizing equipment such as Fragment Analyzer or BioAnalyzer (Agilent) or) and their associated high sensitivity kits
- Qubit® Fluorometer (ThermoFisher Scientific) (optional)

Optional supplies

- Chromatin EasyShear Kit –High SDS (Diagenode, Cat. No. Cat. No. C01020012)
- MicroPlex library preparation kit v3 (Diagenode, Cat. No. C05010001) and corresponding indexes sets for MicroPlex Kit v3

Remarks Before Starting

1. Cell material

This protocol has been optimized for the use of **10,000-100,000 cells** per immunoprecipitation reaction. The lowest recommended cell number per immunoreaction is **10,000 cells**. Three options are possible for chromatin preparation (fixation, cell lysis and chromatin shearing):

- Preparing chromatin from a **BATCH** (option A) of **20,000 to 700,000 cells**. The prepared chromatin can be split into several immunoprecipitation reactions containing a desired cell equivalent in a range of 10,000-70,000 cells per reaction. When possible, this option is preferred in order to limit tube-to-tube variability and cell loss.
- Starting directly from **INDIVIDUAL SAMPLES** (option B) of **10,000 to 100,000 cells**. The prepared chromatin from each individual sample will be used per immunoprecipitation.
- Preparing chromatin from FACS-sorted cells (option C). Cells are fixed as per batch and a desired number of cells (**10,000- 100,000**) are sorted into individual tubes and proceed individually onwards (shearing and immunoprecipitation). The workflow describes the chromatin preparation from cells stained with LIVE/DEAD fixable stain and fixed prior to FACS-sorting. FACS sorting based on cell type–specific markers can be used but it is highly recommended for fix cells prior labelling and FACS to preserve the epigenetic signature. The cells should be sorted out into **25 µl of Lysis Buffer tL1** and the final volume should not exceed **100 µl**. We highly recommend to check with FACS facilities for any specific requirements.

  Please follow a corresponding protocol option.

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 an additional optimization. Usually, the fixation of **8** to **10 minutes** is suitable for most **histone proteins**. Use methanol-free fresh formaldehyde.

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. Note that the chromatin shearing from a limited number of cells is usually quite efficient and does not require a long sonication time.

The reagents included in the kit True MicroChIP-seq Kit allow preparing chromatin using already optimized shearing settings only; it does not contain sufficient reagents for optimization of chromatin shearing.

For optimization of the shearing conditions, we recommend using the Chromatin EasyShear Kit - High SDS (Cat. No. C01020012) which contains all buffers needed for chromatin preparation compatible with the True MicroChIP-seq Kit.

However, it is recommended to check the shearing efficiency for each new chromatin preparation. If chromatin preparation from batch is performed, keep aside an aliquot corresponding to 2,000 cells or higher. If chromatin preparation from individual samples is performed, we recommend including one extra sample to be used per shearing assessment.

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.

DiaMag Protein G-coated magnetic beads (suitable for immunoprecipitation of mouse IgG1, IgG2a, IgG2b and IgG3, rat IgG1, IgG2a, IgG2b and IgG3, rabbit and goat polyclonal Abs and human IgG1, IgG2, IgG3 and IgG4) are available as a separate product (Diagenode, Cat. No. C03010021-220).

5. ChIP-seq grade antibodies

The quality of antibodies used in ChIP-seq is essential for success. It is recommended using 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 and to model the background of the ChIP experiment after sequencing. We recommend including one input per cell type.

7. Controls

The kit contains a negative (IgG) and a positive (H3K4me3) control antibody to monitor the efficiency of the IP on the same sample type as the one used with the antibody of interest. We recommend using the positive control ChIP-seq grade H3K4me3 antibody and the negative control IgG at least once per experiment.

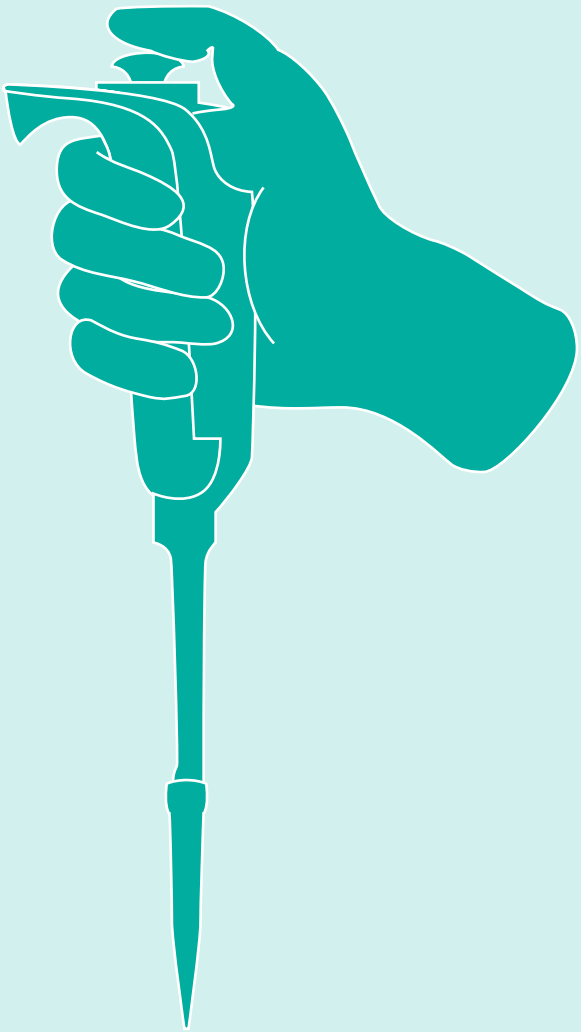
8. DNA purification

The kit contains MicroChIP DiaPure columns for DNA purification allowing an efficient elution of highly concentrated DNA suitable for the down-stream steps (qPCR, DNA quantification and library preparation).

- If the immunoprecipitated DNA will be used for **qPCR analysis** only, we recommend eluting in **50 µl** and use the entire sample for qPCR to determine the enrichment.
- If immunoprecipitated DNA will be used for the **library preparation per sequencing**, the elution volume should be reduced down to **(20)-25 µl** in order to ensure the maximum DNA concentration per library preparation. 10 µl containing minimum 50 pg (or higher when possible) should be used per library preparation using MicroPlex library preparation kit (Diagenode, Cat. No. C05010001). The remaining DNA can be used for DNA quantification (5 µl, optional) and qPCR enrichment check (**10 µl**).
- If no qPCR check neither quantification will be run prior to the library preparation and sequencing, 10 µl elution can be used.

9. Quantitative PCR analysis

We recommend analysing the input and immunoprecipitated samples by SYBR® Green qPCR using **1 positive** and **1 negative control region** to determine the enrichment. The kit contains two primer pairs targeting two human regions which are positive (GAPDH TSS control region) and negative (Myoglobin Exon 2) for the control antibody provided in the kit (H3K4me3 ChIP-seq 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. PCR reactions should be performed at least in duplicate. You can dilute the DNA to perform sufficient PCR reactions. However, ensure that sufficient DNA left for sequencing.



PROTOCOL

OPTION A - BATCH

STEP 1

Cell collection and DNA-protein cross-linking from a batch of cultured cells

 *Day 1*  *30 min. to 1 hour*

The protocol below describes the chromatin preparation starting from a **batch** containing **20,000** up to **1,000,000 cells**. The prepared chromatin can be split for a desired number of immunoprecipitation reactions, each IP being performed with the desired number of cells (from **10,000** to **100,000**). The minimum recommended cell number per batch is **20,000**. This is enough for 2 samples, each of 10,000 cells. The maximum recommended number of cells per batch is 1,000,000. this is enough for 10 samples, each of 100,000 cells. If more cells are needed, then proceed with a separate chromatin preparation.

Determine the number of immunoprecipitation reactions to be run including positive and negative controls and the desired number of cells per immunoprecipitation and start with an appropriated number of cells per batch. An extra amount corresponding minimum to 2,000 cells (or higher) should be added per chromatin shearing assessment.

E.g., if 5 immunoprecipitation reactions will be run each containing of 20,000 cells with chromatin shearing assessment, a batch of 102,000 cells should be used per chromatin preparation.

OPTION A

The final volume of shared chromatin containing the desired amount of cells should be **100 µl** (25 µl of tL1 buffer +75 µl of HBSS).

PBS, cells culture medium and HBSS solution at different temperature will be used.

1.1 Place PBS, cell culture medium and trypsin-EDTA (required for adherent cells) at 37°C.

Place HBSS solution on ice.

Equilibrate a portion of cell culture medium to room temperature.

For adherent cells

1.2 Remove the cell culture medium and rinse the cells with pre- warmed **PBS**. 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.

***NOTE**: The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment may damage the cells. Regularly check if the cells start to detach.*

1.4 Immediately add fresh pre-warmed culture medium to the cells. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 15 ml tube and go directly to point 1.5 of the protocol.

For suspension cells

1.5 Centrifuge for 5 minutes at 500 x g at room temperature and remove the supernatant.

OPTION A

1.6 Resuspend the cells in **1 ml of cell culture medium** (RT) and count the cells. Ensure that you have enough cells accordingly to your experimental design (between 20,000 and 700,000 cells per ml of cell culture medium). Transfer the resuspended cells in a clean 1.5 ml tube.

1.7 Under a fume hood, add **27 µl of 37% formaldehyde** per **1 ml** of **sample**. The final concentration of formaldehyde should be **1%**. Invert tubes immediately 2-3 times to ensure complete mixing.

1.8 Incubate for 8 minutes at room temperature to allow fixation to take place.

1.9 Add **115 µl of Glycine** to the sample. Mix by gentle inversion of the tube 4-5 times. Incubate for 5 minutes at room temperature to stop the fixation. Keep everything at 4°C or on ice from now on.

1.10 Centrifuge samples at 300 x g for 10 minutes at 4°C.

***NOTE**: We recommend the use of a swing-out rotor with soft settings for deceleration.*

1.11 Aspirate slowly the supernatant. Do not disturb the pellet.

1.12 Add **5 µl of protease inhibitor cocktail** to **1 ml** of ice cold **HBSS** and add it to the cell pellet. Invert tubes 4-5 times.

***NOTE**: When working with 100,000 cells and more per batch, you should gently vortex to completely re-suspend the cells.*

1.13 Centrifuge samples at 300 x g for 10 minutes at 4°C.

1.14 Carefully discard the supernatant and keep the cell pellet on ice. Proceed directly to cell lysis.

***NOTE**: We strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP. If not possible, the fixed cells can be stored at -80°C for up to 2 months.*

OPTION A - BATCH

STEP 2

Cell lysis and chromatin shearing of a batch of cells

Day 1

1 to 2 hours

***CAUTION**: Make sure that there are no crystals in the Lysis Buffer tL1 before using it. Gently warm at room temperature and mix until crystals disappear.*

2.1 Prepare **complete Lysis Buffer tL1** and **complete HBSS** by adding the protease inhibitor cocktail 200x (e.g. add 1 µl of protease inhibitor cocktail 200x to 200 µl of Lysis Buffer tL1).

2.2 **25 µl of complete Lysis Buffer tL1** and **75 µl of complete HBSS** will be needed for each individual sample. Scale up the total volume of Lysis Buffer tL1 and HBSS accordingly to the number of immunoprecipitation reactions to be run from the batch. Keep the buffer at room temperature until use.

***NOTE**: e.g., if 5 immunoprecipitation reactions will be run, 125 µl of complete Lysis Buffer tL1 and 375 µl of HBSS will be needed.*

2.3 Add **complete Lysis Buffer tL1** to the cell pellet and resuspend by pipetting.

2.4 Incubate for 5 minutes on ice to ensure complete cell lysis.

2.5 Add **complete HBSS** to the solution and resuspend by pipetting.

OPTION A

***NOTE**: The volume of each individual sample in the batch should be 100 µl (25 µl tL1 and 75 µl HBSS). The total batch volume should be 100 µl x number of individual samples (e.g., if 5 immunoprecipitation reactions will be run, batch volume should be 500 µl).*

Ensure that there are no crystals precipitates in samples. Otherwise, gently warm sample to room temperature until crystals disappear.

2.6 Transfer the cell suspension to sonication microtubes split it into aliquots if needed.

The following tubes can be used for sonication:

Microtubes	Cat. No.	Bioruptor model	Sample volume
0.65 ml	C30010011	Pico	100 µl
1.5 ml with caps	C30010016		100-300 µl
0.5 ml	C30010013	Plus	100 µl
1.5 ml TPX	C30010010-300		100-300 µl

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 3-9 sonication cycles 30'' ON/30'' OFF.
- When using the **Bioruptor Plus**, shear for of 10-20 sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power.

***NOTE**: We recommend performing pilot experiments for each new sample type using the Chromatin easy Shear kit – High SDS (Cat. No. C01020012)*

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. Collect the supernatant which contains the sheared chromatin.

OPTION A

2.9 Use the chromatin for immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months. Avoid freeze/thaw cycles.

***NOTE**: An aliquot of sheared chromatin corresponding to minimum of 2,000 cell equivalent can be used per chromatin shearing assessment. The protocol is described in the "Additional Protocols" section. This step can be omitted when sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimized previously*

OPTION B - INDIVIDUAL SAMPLES

STEP 1

Cell collection and DNA-protein cross- linking from low amounts of cultured cells

1 *Day 1* *30 min. to 1 hour*

The protocol below describes the chromatin preparation from individual samples from 10,000 up to 100,000 cells. The chromatin can then be used directly for one immunoprecipitation reaction.

If a chromatin shearing assessment will be performed, we recommend including one extra sample.

> ***NOTE**: PBS, cells culture medium and HBSS solution at different temperature will be used.*
>
> *Pre-warm PBS, cell culture medium and trypsin-EDTA (required for adherent cells) at 37°C.*
>
> *Place HBSS solution on ice." should start from the new line. For an example please look at the page 17, point 1.1*
>
> *Equilibrate a portion of cell culture medium to room temperature.*

OPTION B

For adherent cells

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

1.2 Remove the medium and rinse the cells with pre-warmed PBS. 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.

***NOTE**: 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 pre-warmed culture medium. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 1.5 ml tube.

For suspension cells

Collect suspension cells in a 1.5 ml tube and go directly to point B1.5 of the protocol.

1.5 Centrifuge for 5 minutes at 500 x g (at room temperature) and remove the supernatant.

1.6 Resuspend the **cells in cell culture** medium (room temperature) and count them using a cell counter.

1.7 Distribute cell suspension containing the desired cell number to individual tubes.

OPTION B

If 0.5/0.65 ml sonication tubes and accessories are available, distribute cells to sonication microtubes of choice:

- 0.65 ml microtubes (Cat. No. C30010011) associated with Bioruptor Pico
- 0.5 ml microtubes (Cat. No. C30010013) associated with Bioruptor Plus

***NOTES**: these tubes will be kept through the cell fixation, lysis and shearing reducing the sample loss (no sample transfer)*

If 0.5/0.65 ml sonication tubes and accessories are not available, distribute cells to standard 1.5 ml tubes.

***NOTES**: these tubes will be kept through the cell fixation, lysis but sample should be transferred into appropriate sonication tubes prior to the shearing.*

1.8 Add **cell culture medium** to reach a final volume of **100 µl** in each tube. Under a fume hood, add **2.7 µl of 37% formaldehyde** to each tube containing 100 µl of cell suspension and mix gently

1.9 Incubate 8 minutes at room temperature with occasional manual agitation 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 **11.5 µl of Glycine** to the cells to stop the fixation. Mix gently. Incubate for 5 minutes at room temperature to stop the fixation. Keep everything at 4°C or on ice from now on.

1.11 Collect the cells by centrifugation at 300 x g for 10 minutes at 4°C.

***NOTE**: We recommend the use of a swing-out rotor with soft settings for deceleration.*

OPTION B

1.12 Aspirate the supernatant slowly. Do not disturb the pellet.

1.13 Prepare **complete HBSS Buffer** by adding the protease inhibitor cocktail 200x. 120 µl of HBSS is required per one individual sample. Scale accordingly.

1.14 Wash the cross-linked cells with 120 µl of ice-cold complete HBSS as follows:

- Add **120 µl of complete HBSS**
- Gently mix to resuspend the cells
- Centrifuge at 300 x g for 10 minutes at 4°C (in a swing-out rotor with soft settings for deceleration). Aspirate slowly the supernatant. Do not disturb the pellet.

OPTION B - INDIVIDUAL SAMPLES

STEP 2

Cell lysis and chromatin shearing of a batch of cells

Day 1 *1 to 2 hours*

2.1 Shear the chromatin by sonication using the Bioruptor. If sample were proceeded using standard 1.5 ml tubes, transfer into an appropriated sonication tubes adapted for your device.

Microtubes	Cat. No.	Bioruptor model	Sample volume
0.65 ml	C30010011	Pico	100 µl
1.5 ml with caps	C30010016		100-300 µl
0.5 ml	C30010013	Plus	100 µl
1.5 ml TPX	C30010010-300		100-300 µl

2.2 Choose the protocol which is adapted to your device:

- When using the **Bioruptor Pico**, shear for 3-9 sonication cycles 30'' ON/30'' OFF.
- When using the **Bioruptor Plus**, shear for of 10-20 sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power.

***NOTE**: We recommend performing pilot experiments for each new sample type using the Chromatin easy Shear kit – High SDS (Cat. No. C01020012)*

OPTION B

2.3 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. Collect the supernatant which contains the sheared chromatin into individual 1.5 ml tubes.

2.4 Use the chromatin for immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months. Avoid freeze/thaw cycles.

***NOTE**: An aliquot of sheared chromatin corresponding to minimum of 2,000 cell equivalent can be used per chromatin shearing assessment. The protocol is described in the "Additional Protocols" section. This step can be omitted when sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimized previously*

OPTION C - FACS-sorted cells

STEP 1

Cell collection and DNA-protein cross-linking from FACS-sorted cells

Day 1 *30 min. to 1 hour*

The protocol below describes the preparation of **chromatin from FASC-sorted cells**.

Cells are fixed from a batch and a desired number of cells (**10,000-70,000**) are FACS-sorted directly to Lysis Buffer tL1 and proceed individually onwards. Cells can be (optionally) stained prior to the fixation with LIVE/DEAD fixable Aqua stain (or similar) to exclude dead cells during the sorting.

Determine the number of immunoprecipitation reactions to be run including positive and negative controls and the desired number of cells per immunoprecipitation and start with an appropriated number of cells per batch. We recommend including one extra sample per chromatin shearing assessment.

We recommend checking the sheath fluid volume for a desired number of sorted cells prior starting the experiment. Ensure that the final volume (sorted cells plus 25 µl of Lysis Buffer tL1) does not exceed **100 µl**.

OPTION C

The protocol below describes the chromatin preparation from a batch of up to 1 million cells.

Attention PBS, cells culture medium and HBSS solution at different temperature will be used.

1.1 Pre-warm PBS, cell culture medium and trypsin-EDTA (required for adherent cells) at 37°C.

Place HBSS solution on ice.

Equilibrate a portion of cell culture medium to room temperature.

For adherent cells

1.2 Remove the cell culture medium and rinse the cells with pre-warmed PBS. 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.

***NOTE**: The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment 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 15 ml tube and go directly to point C1.5 of the protocol.

For suspension cells

1.5 Collect suspension cells in a 1.5 ml tube and go directly to point 1.5 of the protocol.

1.6 Centrifuge for 5 minutes at 500 x g at room temperature and remove the supernatant.

OPTION C

1.7 Resuspend the cells in **cold PBS** in a clean 1.5 ml tube and count the cells. Adjust the cell density to have approximately **1 million cells per 1 ml PBS**.

1.8 (Optional) Add aqua dye (1 µl per 1 ml cell suspension) and stain for 30 minutes on ice.

1.9 Centrifuge samples at 300 x g for 10 minutes at 4°C and remove the supernatant. Do not disturb the pellet.

1.10 Resuspend cells in **1 ml of ice-cold PBS** and centrifuge samples at 300 x g for 10 minutes at 4°C and remove the supernatant.

1.11 Under a fume hood, add **27 µl of 37% formaldehyde** per **1 ml** of room-temperature **PBS**. The final concentration of formaldehyde should be **1%**.

1.12 Resuspend the cell pellet sample in this solution. Invert tubes immediately 2-3 times to ensure complete mixing.

1.13 Incubate for 8 minutes at room temperature to allow fixation to take place.

1.14 Add **115 µl of Glycine** to the sample. Mix by gentle inversion of the tube 4-5 times. Incubate for 5 minutes at room temperature to stop the fixation. Keep everything at 4°C or on ice from now on. Centrifuge samples at 300 x g for 10 minutes at 4°C.

***NOTE**: We recommend the use of a swing-out rotor with soft settings for deceleration.*

1.15 Add **200 µl** of **5% BSA** to **1 ml of ice-cold PBS**. Aspirate slowly the supernatant and re-suspend cells in 1 ml PBS/BSA.

OPTION C

1.16 Filter the cell suspension using an appropriated cell strainer (<80 µm) accordingly to the supplier recommendations.

***NOTE**: It is essential to filter all samples before reading them in a Flow Cytometer. During processing, cells will pass through a nozzle. Clumps and debris can clog the instrument fluidics and either distort the measurements or obstruct them completely.*

1.17 Sort a desired number of cells (10,000 – 70,000) into **25 µl of complete Lysis Buffer tL1** supplemented with **protease inhibitors cocktail**. Ensure that the final volume (sorted cell and Lysis Buffer tL1) does not exceed **100 µl**. Adjust the final volume to 100 µl using complete HBSS.

***NOTE**: If the sheath fluid volume is not negligible, cells can be sorted into PBS, centrifuged at 300 x g for 10 minutes at 4°C and resuspended in 25 µl of Lysis Buffer tL1.*

OPTION C - FACS-sorted cells

STEP 2

Cell lysis and chromatin shearing of a batch of cells

Day 1 *1 hour*

2.1 Transfer the cell suspension to sonication microtubes. The following tubes can be used for sonication:

Microtubes	Cat. No.	Bioruptor model
0.65 ml	C30010011	Pico
1.5 ml with caps	C30010016	
0.5 ml	C30010013	Plus
1.5 ml TPX	C30010010-300	

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

- When using the **Bioruptor Pico**, shear for 3-9 sonication cycles 30'' ON/30'' OFF.
- When using the **Bioruptor Plus**, shear for of 10-20 sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power.

***NOTE**: We recommend performing pilot experiments for each new sample type using the Chromatin easy Shear kit – High SDS (Cat. No. C01020012)*

OPTION C

2.3 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. Collect the supernatant which contains the sheared chromatin.

2.4 Use the chromatin for immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months. Avoid freeze/thaw cycles.

***NOTE**: An aliquot of sheared chromatin corresponding to minimum of 2.000 cell equivalent can be used per chromatin shearing assessment. The protocol is described in the "Additional Protocols" section. This step can be omitted when sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimized previously*

FOR OPTION A, B & C

STEP 3

Magnetic immunoprecipitation

1-2 *Day 1-2* *1 hour overnight incubation, 1.5 hours*

3.1 Determine the number of IP reactions to be run including the negative and positive control IPs. Take the required amount of DiaMag Protein A-coated magnetic beads and transfer it to a clean 1.5 ml tube. **10 µl of beads are required per IP**.

3.2 Wash the beads 4 times with **50 µl of ice-cold Beads Wash Buffer tBW1** per IP. To wash the beads, add tBW1, resuspend the beads by pipetting up and down several times and place the tubes in the DiaMag1.5 magnetic rack. Wait for 1 minute to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 3 times.

3.3 After the last wash, resuspend the beads in **Beads Wash Buffer tBW1** adding the original volume of beads (this means **10 µl** per IP).

3.4 Prepare the **immunoprecipitation mix** in 1.5 ml tubes as described in the table below.

Component	Volume per reaction
Shared chromatin	100 µl
ChIP Buffer tC1	100 µl
200x Protease Inhibitor Cocktail (black cap)	0.75 µl

3.5 Set aside **20 µl** to be used as an **input sample** and keep at 4°C.

***NOTE**: If ChIP is run using the same type of sample (cell line, treatment etc) 1 input will be enough. It can be taken from negative IgG control sample. If ChIP is run using different type of sample (e.g. different treatments or different cell type), individual input should be taken from each sample.*

3.6 Add the specific **antibody** to each tube.

***NOTE**: The required amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody. If a positive control IP is included, use 0.5 µg of the H3K4me3 positive control antibody). If a negative control IP is included, use 0.5 µg of Rabbit IgG.*

3.7 Add **10 µl** of the **washed magnetic beads** to each tube.

3.8 Incubate overnight at 4°C on a DiaMag Rotator.

3.9 Perform the washes as follows: briefly spin the tubes and place them in 1.5 ml Diagenode magnetic rack (Cat. No. B04000003). Wait for 1 minute and remove the supernatant. Add **100 µl of Wash Buffer tW1**, gently shake the tubes to resuspend the beads and incubate for 5 minutes on the DiaMag rotator at 4°C.

3.10 Repeat the washing step as described above once with **Wash Buffer tW2**, **tW3** and **tW4**, respectively.

FOR OPTION A, B & C

STEP 4

Elution, decross-linking and DNA purification

2 *Day 2* *5 hours*

***NOTE**: Before the first use of the kit, 2**4 ml of ethanol** must be added to **6 ml** of the **DNA Wash Buffer**. Never leave the bottle open during storage to avoid evaporation*

4.1 After removing the last wash buffer, add **100 µl of Elution Buffer iE1** to the beads, resuspend the beads pellet and incubate for 30 minutes on the DiaMag rotator at room temperature.

4.2 Briefly spin the tubes and place them in the magnetic rack. Transfer the supernatant to a new tube and add **4 µl of Elution Buffer tE2**. At the same time, add **80 µl of Elution Buffer iE1** and **4 µl of Elution Buffer iE2** to the **20 µl INPUT sample**. Incubate for 4 hours (or overnight) in a thermomixer at 1300 rpm and 65°C.

***NOTE**: If precipitation is observed in Elution Buffer iE1, warm it at 37°C until it becomes clear.*

4.3 Briefly spin the tubes and add **500 µl ChIP DNA Binding buffer** to each sample. Mix briefly.

4.4 Transfer mixture to a provided Spin column in a Collection tube.

4.5 Centrifuge at ≥ 10,000 x g for 30 seconds and discard the flow-through.

4.6 Add **200 µl DNA Wash buffer** to the column. Centrifuge at ≥ 10,000 x g for 30 seconds. Repeat wash step one more time.

4.7 Transfer the column to a new 1.5 ml microcentrifuge tube and add **10-50 µl DNA Elution buffer** directly to the column matrix. Centrifuge at ≥ 10,000 x g for 30 seconds to elute the DNA.

- If the immunoprecipitated DNA will be used for **qPCR analysis** only, elute in **50 µl** and use the entire sample for qPCR to determine the enrichment.
- If the immunoprecipitated DNA will be used per **sequencing with no check by qPCR**, elute in **10 µl** and use the entire sample per library prep using MicroPlex Kit (Cat. No. C05010001)
- If immunoprecipitated DNA will be used per **sequencing with qPCR enrichment check**, elute in **20 µl**. Keep 10 µl per library preparation and use the remaining 10 µl for qPCR to determine the enrichment.
- If the immunoprecipitated DNA will be quantify (optional), add **5 µl** to the desired elution volume. Use 5 µl aliquot and quantify using Qubit® High sens kit (ThermoFisher).

FOR OPTION A, B & C

STEP 5

Quantitative PCR analysis

Day 2

2 to 3 hours

5.1 Determine the total number of regions to be analyzed by qPCR for each sample. Dilute immunoprecipitated DNA and a corresponding input accordingly to number of PCR reactions if needed. Take into account that **5 µl** will be used per **one PCR reaction** with duplicates.

5.2 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 immunoprecipitated or INPUT DNA

5.3 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		

**Carefully check 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*

5.4 Record the threshold cycles (Ct values) from the exponential phase of the qPCR for the immunoprecipitated DNA sample and input for each primer pair.

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

$$\% \text{ recovery} = 2^{((Ct_{input} - 3.32) - Ct_{sample})} * 100\%$$

- Ct_{sample} and Ct_{input} are the threshold cycles from the exponential phase of the qPCR for the immunoprecipitated DNA sample and input, respectively.
- 2 is the amplification efficiency
- 3.32 is a compensatory factor to account for the 10x dilution of input sample (20 µl out of 200 µl) corresponding to log2(10).

***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.*

If an expected enrichment over positive loci with a low background is observed, proceed to the library preparation using an appropriate protocol (not supplied with True Micro ChIP kit).

For library preparation compatible with Illumina® sequencers, we highly recommend using Diagenode MicroPlex Library Preparation Kits v3 (Cat. No. C05010001).

For flexibility of the choice different formats of compatible primer indexes are available separately:

Dual indexes:

- C05010003 - 24 Dual indexes for MicroPlex Kit v3 /48 rxns
- C05010004 - 96 Dual indexes for MicroPlex Kit v3 – Set I /96 rxns
- C05010005 - 96 Dual indexes for MicroPlex Kit v3 – Set II /96 rxns
- C05010006 - 96 Dual indexes for MicroPlex Kit v3 – Set III /96 rxns
- C05010007 - 96 Dual indexes for MicroPlex Kit v3 – Set IV /96 rxns

Unique dual indexes

- C05010008 - 24 UDI for MicroPlex Kit v3 - Set I
- C05010009 - 24 UDI for MicroPlex Kit v3 - Set II

Example of Results

Chromatin preparation and immunoprecipitation analysis

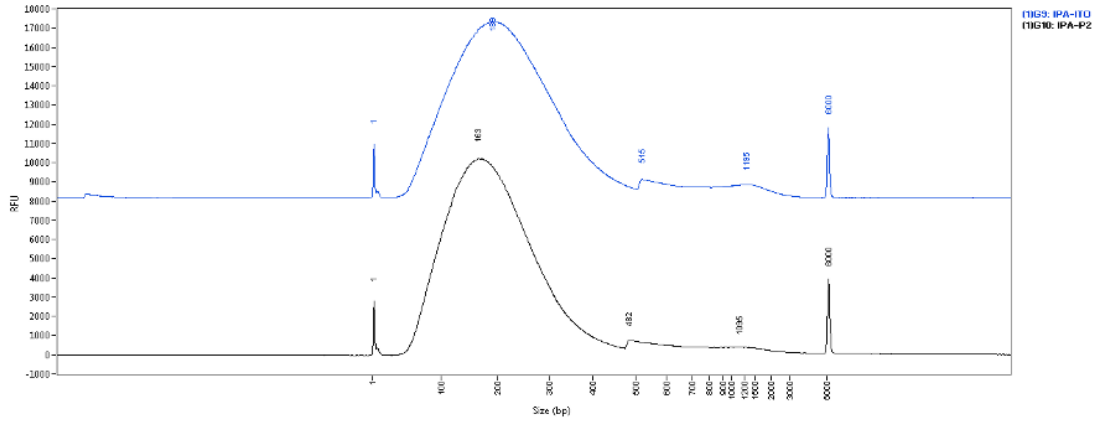


Figure 1. Size distribution of purified DNA from de-crosslinked chromatin. Chromatin from K562 cells was prepared accordingly to the manual (batch protocol with 25,000 cells per one sample) and sheared using the Bioruptor Pico for 5 cycles (30'' On/30''Off). Chromatin was de-crosslinked and purified and fragment size was assessed using the Fragment Analyzer (Agilent).

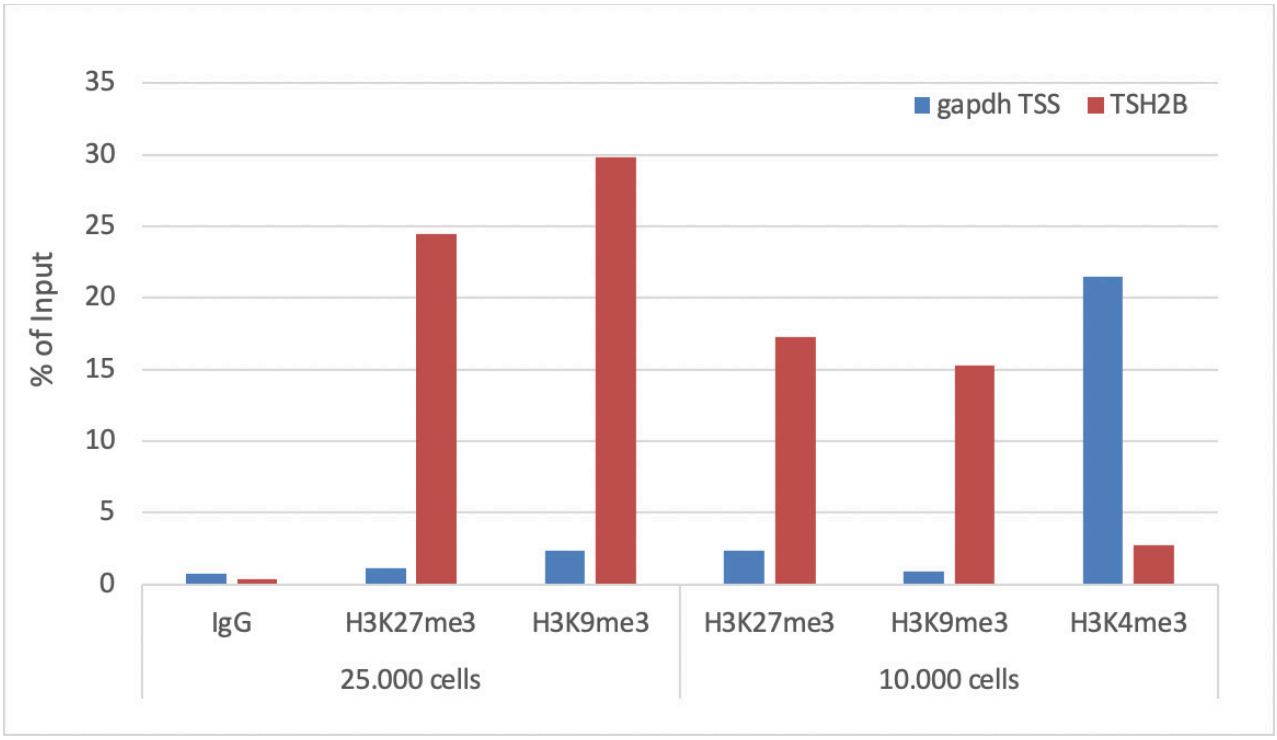
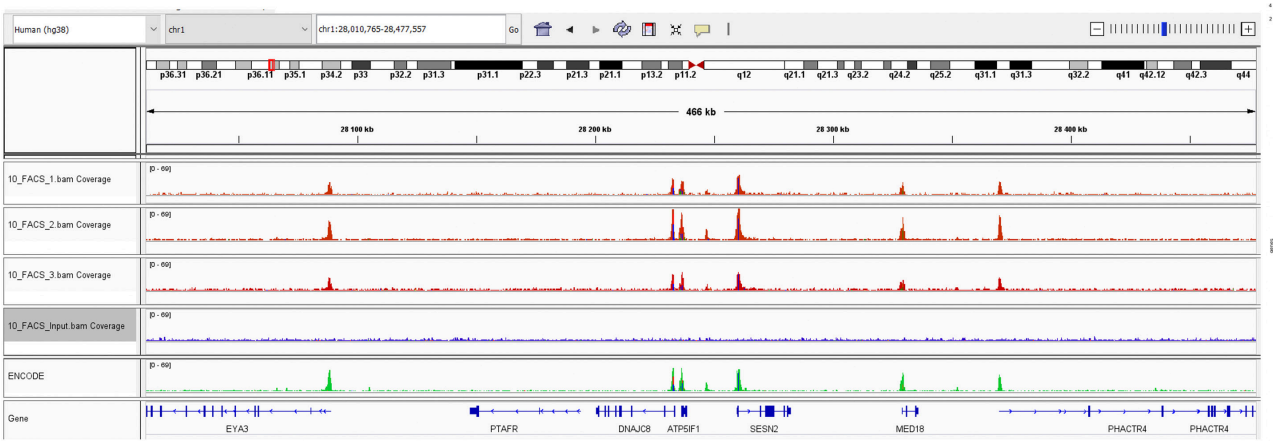


Figure 2. Chromatin immunoprecipitation analysis from K562 cells using control H3K4me3 antibody, H3K27me3 (Cat. No. C15410195) and H3K9me3 (Cat. No. C15410193) antibodies. ChIP was performed from 25,000 or 10,000 cells including negative IgG control antibodies as indicated. Quantitative PCR was performed with the control GAPDH TSS promoter (active gene) and the TSH2B (inactive gene) (Cat. No. C17011041) primer sets. The recovery is expressed as a percent of input. An expected enrichment over GAPDH TSS promoter is observed with H3K4me3 (active chromatin mark) while H3K27me and H3K9me3 are enriched over inactive region.

A.



B.

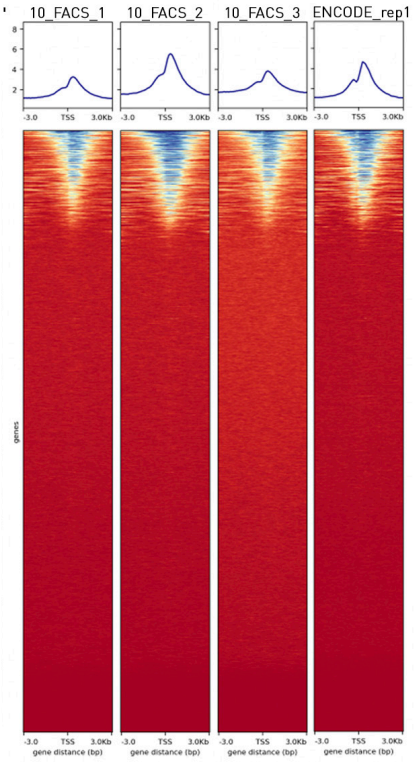


Figure 3. Successful chromatin profiling from 10.000 of FACS-sorted cells

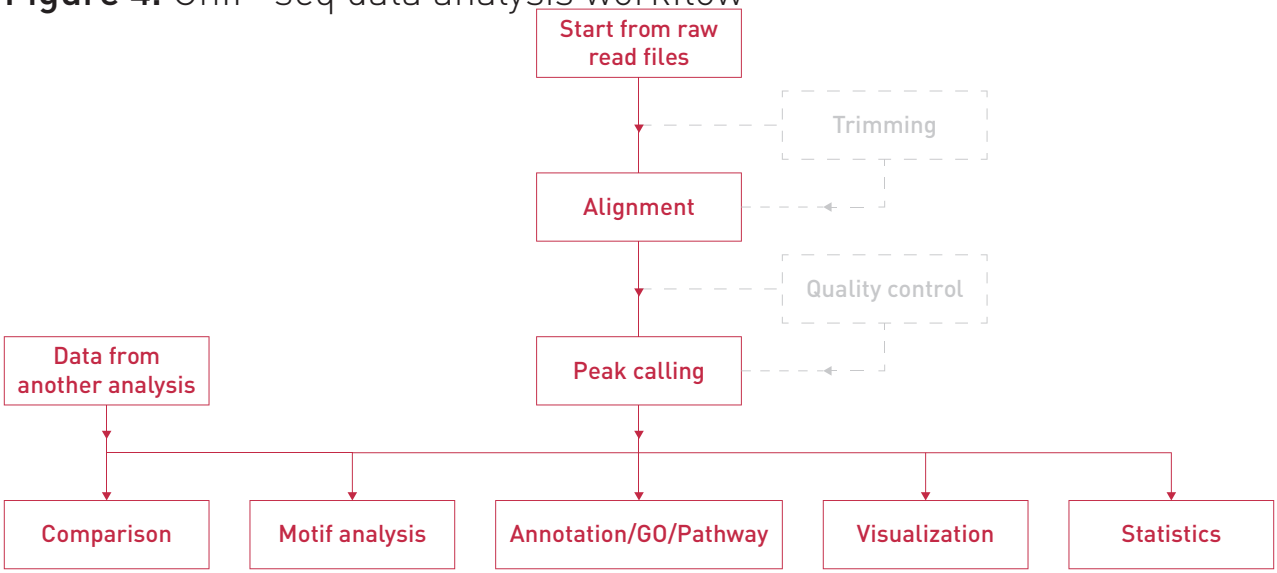
(A) Integrative genomics viewer (IGV) visualization of ChIP-seq experiments and heatmap 3kb upstream and downstream of the TSS (B) for H3K4me3. ChIP has been performed using 10.000 of FACS-sorted cells (K562) and H3K4me3 antibody (C15410003) accordingly to True MicroChIP-seq protocol followed by the library preparation using MicroPlex Library Preparation Kit (C05010001). Data were compared to ENCODE standards.

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.

Figure 4. ChIP-seq data analysis workflow



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 your biological questions. Several options and tools are available for further analyses. Your project goals will determine which ones you 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 are in them, the number of peaks, mean size, significance, etc. These figures are also very useful for comparing datasets.

a. Peak callers usually provide per peak and/or summary statistics after peak detection
b. HOMER
c. GREAT
d. BEDTools

7. **Motif search**: For transcription factors, data peaks frequently occur at specific motifs, though some HM peaks can also lean toward certain sequence patterns. Therefore identifying these motifs and checking their enrichments over them is a good practice for TF data analysis, which is 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 with 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 optimal 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. DiffBin

ADDITIONAL PROTOCOLS

Protocol For Chromatin Shearing Analysis

General remarks

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.

> ***NOTE**: Before the first use of the kit, 24 ml of ethanol must be added to 6 ml of the DNA Wash Buffer. Never leave the bottle open during storage to avoid evaporation*

Reverse cross-linking

1. Take an aliquot, containing the required amount of sheared chromatin:

   > ***NOTE**: an equivalent of 1,000-2,000 cells (or higher) should be used in a combination with a microfluidic device (Fragment Analyzer or Bioanalyzer) for size assessment.*

2. Adjust, if needed, the volume of the sheared chromatin to 100 µl using **TE buffer**.

3. Add **100 µl of the Elution Buffer tE1** and **8 µl of Elution Buffer tE2** to each sample. Mix thoroughly and incubate samples at 65°C for 4 hours (or overnight) in a thermoshaker at 1300 rpm.

4. Purify DNA using MicroChIP DiaPure Columns. Add 5**00 µl of ChIP DNA Binding Buffer** to each sample and mix briefly.

5. Transfer the mixture to a provided spin column in a collection tube and centrifuge at ≥ 10,000 x g for 30 seconds. Discard the flow-through.

6. Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at ≥ 10,000 x g for 30 seconds.

7. Add **1 µl of RNase cocktail** (not provided with the kit) directly to the center of the spin column membrane and incubate for 15 minutes at room temperature.

8. Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at ≥ 10,000 x g for 30 seconds.

9. Add **6 µl of DNA Elution Buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥ 10,000 x g for 30 seconds to elute the DNA.

   ***NOTE**: Up to 50 µl of DNA Elution Buffer can be used if less concentrated DNA is required for analysis.*

Fragment size assessment

10. Analyze the purified DNA using Fragment Analyzer or Bioanalyzer and their high sensitivity kits accordingly to the manufacture's recommendation.

FAQs

Is the included control H3K4me3 antibody compatible with mouse?

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

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

10 µl of DiaMag Protein A-coated magnetic beads can bind 3 µg of antibody.

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, especially when ChIP is staring with a low number of cells. Avoid using an excess of antibody which will lead to a high background. If the antibodies are selected from a source other than Diagenode, 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.

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 specificity of protein A-coated magnetic beads?

The Kit 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).

I do not have a Fragment Analyzer or BioAnalyzer. How can I assess the shearing?

The conventional agarose gel is not sensitive enough to visualize low amounts of DNA. If chromatin shearing assessment is performed using agarose gel electrophoresis, a minimum input of 40,000-50,000 cells is required for reliable assessment. Therefore, several replicates, equivalent to 40,000-50,000 cells, should be pooled before loading onto agarose gel. We recommend using a thin agarose gel for better signal visualization. Both the pre- and post-staining of the agarose gel with ethidium bromide or SybrSafe dye can be used for visualization of sheared fragments. Some slight differences might be observed between post- and pre-stained gels. Post-staining eliminates any possibility that the dye interferes with the migration and ensures an even background noise. However, pictures are usually less clear and bright with some background noise. If pre-stained agarose gels are used, it is advised that the electrophoresis buffer contains the stain in the same concentration as in the gel. If the stain is present in the gel but not in the buffer, the gel will result in uneven staining because the free dye migrates towards the top of the gel leaving the bottom part with no stain. Therefore, the background noise becomes non-uniform.

What is the difference between the kit True MicroChIP-seq, C01010132 et the True MicroChIP Kit, C01010130?

The kit True MicroChIP-seq is an upgraded version of the kit True Micro ChIP: **1)** the MicroChIP DiaPure columns for DNA purification has been included in the kit, **2)** new protocols, inlcuding the FACS sorted cells, have been validated.

Related Products

Product	Reference
Chromatin EasyShear kit – High SDS	C01020012
Bioruptor Pico	B01080010
MicroPlex Library Preparation kit v3	C05010001
24 UDI for MicroPlex v3 - Set I	C05010008
24 UDI for MicroPlex v3 - Set II	C05010009

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

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