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Pre-ChIP Assay Preparation Guidelines for ChIP Kit (Low cell number) ab270814

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ChIP Kit (Low cell number) datasheet:

www.abcam.com/ab270814

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This product is for research use only and is not intended for diagnostic use.

Please read before performing assay

Cell Number and sample manipulation

This protocol has been optimized for shearing of 10 000 cells in 100 µl using the sonicator and then subsequent immunoprecipitation on 10,000 cells in 200 µl. Determine the number of IP you will perform and start with fixation of a unique batch of chromatin. For example, if you would like to perform 4 ChIP on the same chromatin, start with fixation of 40,000 cells. Add also an extra chromatin preparation to use for the input.

Due to the low amount of starting material it is critical to avoid sample loss throughout the experiment to ensure reproducible and consistent results. Avoid pipetting up and down when adding buffers to samples. It is also recommended to use low retention Eppendorf tubes at each step of the protocol to minimize sample lost.

The use of an automated cell counter is also recommended to reduce variations in the amount of the starting cell number.

This kit is also compatible with higher cell numbers. Efficient shearing has been validated with the kit in a cell range from 10,000 cells to 100,000 cells to allow performing ChIP assay on 10,000 to 100,000 cells. Determine the number of cells you would like to use per ChIP reaction (between 10,000 and 100,000 cells) and perform shearing on that cell number. Fixation can be done on larger cell numbers (scale accordingly volumes of Lysis Buffer L1 and HBSS to use) and cell lysate will then be split into 100 µl aliquots (corresponding to the number of cells that will be use per IP reaction) before shearing. Then sheared chromatin will be diluted two times with ChIP Buffer C1 before performing the immunoprecipitation.

Shearing optimization and sheared chromatin analysis

Before starting the ChIP, the chromatin should be sheared to fragments in the 100 to 600 bp range. The shearing conditions mentioned in the protocol are adequate for a variety of cell types. However, you should optimize shearing conditions for your specific cell type and fixation protocol before starting a ChIP. Nevertheless analysis of shearing efficiency is not obvious when working with 10,000 cells due to the low amount of DNA recovered after sonication and crosslinking reversion for subsequent analysis on agarose gels. Therefore at least 6 replicates should be performed to check the shearing efficiency and pooled before loading onto agarose gel.

Antibodies

The optimal amount of antibody to use per ChIP experiment has to be optimized for each antibody. However, we recommend to start with 0.25 µg of antibody per IP when performing ChIP on 10,000 cells. The kit contains a negative (IgG) and a positive (H3K4me3) control antibody. We recommend including one IgG negative IP control in each series of ChIP reactions. We also recommend using the positive control ChIP-seq grade H3K4me3 antibody at least once. The kit also contains human qPCR primer pairs for amplification of a positive and negative control target for H3K4me3 (GAPDH-TSS and Myoglobin exon 2, respectively).

Magnetic Beads

This kit includes 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.**

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. If the antibody of interests belongs to a different class of immunoglobulins (mouse IgG1 and IgG3, rat or goat polyclonal Abs, and human IgG3), Protein G-coated magnetic beads should be used instead.

Δ Note: 20 µl of Protein A-coated magnetic beads can bind 7 µg of antibody.

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.

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 nonspecific 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.

Input

The input sample corresponds to whole DNA which went through the full ChIP procedure without any immunoselection. The input sample is used as a reference to calculate the recovery at the end of the ChIP procedure. It is also used by most of the bioinformatics tools for analysis of ChIPseq data where it serves to determine the bias which may result from experimental conditions. We recommend including one input for each series of ChIP reactions.

Quantification

Determine the concentration of the IP'd DNA after the ChIP with a highly sensitive method such as the 'Quant-IT dsDNA HS assay kit' on the Qubit system. PicoGreen is also suitable but UV spectrophotometric methods such as the NanoDrop are usually not sufficiently sensitive. In most cases it is sufficient to use approximately one quarter of the IP'd material for quantification (when working with 10,000 cells). The expected DNA yield will be dependent on different factors such as the cell type, the quality of the antibody used and the antibody target.

Quantitative PCR

Before sequencing the samples, we recommend analysing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. In order to have sufficient DNA left for sequencing, we recommend not using more than one third of the total IP'd DNA for qPCR. You can dilute the DNA (1/4 or more) to perform sufficient PCR reactions. PCR reactions should be performed at least in duplicate although performing them in triplicate is recommended to be able to identify potential outliers.

Quantitative PCR data interpretation

The efficiency of chromatin immunoprecipitation of particular genomic loci can be expressed as the recovery of that locus calculated as the percentage of the input (the relative amount of immunoprecipitated DNA compared to input DNA).

% recovery =100* 2^[(Ct(input) – log(X%)/log2) – Ct(sample)]

- Ct (sample) and Ct (input) are threshold values obtained from exponential phase of qPCR for the IP'd DNA sample and input sample respectively
- (logx%/log2) accounts for the dilution 1/x of the input.

If the amount used for the input was 10% of the amount used for ChIP, the recovery can be calculated as follows:

% recovery =100* 2^[(Ct(input) – 3.32) – Ct(sample)]

This equation assumes that the PCR is 100% efficient (amplification efficiency = 2). For accurate results the real amplification efficiency, if known, should be used.
Criteria to decide whether the sample is good enough for sequencing will be largely target dependant. Therefore, the following are only general guidelines:
• the recovery of the positive control target should be at least 5%
• the ratio of the positive versus the negative control target should be at least 5

Technical Support

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