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# Pre-ChIP Assay Preparation Guidelines for ChIP Kit (Transcription factors, ChIP-seq) ab270813

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ChIP Kit (Transcription factors, ChIP-seq) datasheet:

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# Please read before performing assay

## Cell Number

The protocol describes the preparation of a batch of chromatin from approximately 25 million cells which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 4 million cells per IP reaction are used in this standard protocol. The protocol is optimized for the use of 250 µl of sheared chromatin in a total volume of ChIP reaction equal to 350 µl. It is crucial to keep these volumes consistent for optimal results.

Please note that the described protocol uses a prompt direct fixation in a cell culture plate. Fixed and scraped cells cannot be accurately counted. This means that for adherent cells you need to use an approximate estimation of cell number per plate. Alternatively, an additional parallel plate for counting can be prepared. Suspension cells can be counted before the fixation.

Depending on the abundance of the target, the specificity of the antibody, and the amount of cells available, it may be possible to scale up and down the amount of cells per IP and/or start with a smaller or a bigger batch of cells.

For using lower amounts of cells per IP, you can start with a batch of 25 millions of cells (as for a standard protocol) and follow the protocol up to the chromatin shearing step. Then simply dilute the sheared chromatin in shearing buffer S1b before adding it to the IP reaction. The final volume of diluted chromatin containing the desired amount of cells should be 250 µl per IP reaction.

If starting with an amount of cells different from the standard protocol or if you want to use a higher amount of cells per IP, 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 L1b and L2 buffers using 1 ml of L1b and 0.6 ml of L2b per 1 million cells. Define the volume of shearing buffer iS1b taking into account that you will need:

- 250 µl of sheared chromatin (containing a desired amount of cells) per IP reaction
- 2.5 µl of sheared chromatin per input
- 50 µl of sheared chromatin for chromatin shearing assessment

• Add 5% excess of S1b

Resuspend the cells in the required volume of shearing buffer S1b 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.

## Tissue Amount

The protocol describes the preparation of a batch of chromatin from approximately 200 mg of tissue which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 30 mg of tissue per IP reaction are used in this standard protocol. The protocol is optimized for use of 250 µl of sheared chromatin in a total volume of ChIP reaction equal to 350 µl. It is crucial to keep these volumes constant for optimal results.

Depending on the abundance of the target, the specificity of the antibody and the amount of tissue available, it may be possible to scale up and down the amount of tissue per IP and/or start with a smaller or a bigger batch of tissue.

For using lower amounts of tissue per IP, start with 200 mg of tissue (as for a standard protocol) and follow the protocol up to the chromatin shearing. Then simply dilute the sheared chromatin in shearing buffer S1b before adding it to the IP reaction. The final volume of diluted chromatin containing a desired amount of tissue should be 250 µl per IP reaction.

If starting with a tissue amount different from the standard protocol or if you want to use a higher amount of tissue per IP, first determine the amount of tissue that you will use per IP and the total number of IPs.

Fix the cells as described in the standard protocol. Follow the standard protocol for tissue fixation, collection and lysis. Do not scale lysis buffers L1b and L2. Define the volume of shearing buffer iS1b taking into account that you will need:

• 250 µl of sheared chromatin (containing the desired amount of tissue) per IP reaction

• 2.5 µl of sheared chromatin per input

• 50 µl of sheared chromatin for chromatin shearing assessment

• Add 5% excess of S1b

Resuspend the tissue in the required volume of shearing buffer S1b and follow the standard protocol.

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

When harvesting cross-linked chromatin from tissue samples, the yield of chromatin can vary significantly between tissue types. Usually, the amount of chromatin to be used per IP is 3-10 µg. We recommend performing a pilot experiment to determine the optimal amount of tissue. Once determined, it should be kept consistent between experiments.

## 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 between 10 and 20 minutes). Please note that a longer fixation may lead to chromatin resistant to sonication.

However, for higher order and/or dynamic interactions, other crosslinkers should be considered for efficient protein-protein fixation.

## 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.

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.

## 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 chromatin preparation.

## Positive and Negative Controls

The kit contains a negative (IgG) and a positive (CTCF) 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. We also recommend using the positive control ChIP-seq grade CTCF antibody at least once.

The kit also contains qPCR primer pairs for amplification of a positive and negative control target for CTCF in Human (H19 imprinting control region and Myoglobin exon 2, respectively).

## Quantification

After the ChIP, determine the concentration of the IP'd DNA 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. In most cases it is sufficient to use approximately 10% of the IP'd material for quantification. The DNA yield will be dependent on different factors such as cell type, quality of the antibody used and antibody target. The expected DNA yield obtained with the positive control CTCF antibody on 4,000,000 HeLa cells is approximately 20 ng.

## 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 (H19 imprinting control region) and negative (Myoglobin Exon 2) for the control antibody provided in the kit (CTCF 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. In order to have sufficient DNA left for sequencing, we recommend not using more than 10% of the total IP'd DNA for qPCR. You can dilute the DNA (1/10 or more) 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.

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