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Pre-ChIP Assay Preparation Guidelines for ChIP Kit (Histones, ChIP-seq) ab270812

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ChIP Kit (Histones, 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 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 in a total volume of ChIP reaction equal 300 µl. It is crucial to keep these volumes consistent for optimal results.

Depending on the abundance of the target, the specificity of the antibody, and the number of cells available, it may be possible to scale down to 100,000 cells as described below. Scaling up is usually not necessary but if a higher yield of IP'd DNA is required, we recommend performing separate ChIPs and pool the IP'd DNA before purification. For using lower numbers of cells per IP:

- You can start with a batch of 7 million of cells follow the protocol up to the chromatin shearing step. Then simply dilute the sheared chromatin in the shearing buffer S1 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.

- You can start with a smaller batch of cells. 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 down the volume of L1 and L2 buffers by 1 ml of L1 and 1 ml of L2 per 1 million cells. Define the volume of shearing buffer S1 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 S1

Resuspend the cells in the required volume of shearing buffer S1 and follow the standard protocol.

Please note that changing 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 30-40 mg of tissue which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 5 mg of tissue per IP reaction are used in this standard protocol. The protocol is optimized for use of 100 µl of sheared chromatin in a total volume of ChIP reaction equal 300 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 down to 1.5 mg of tissue per IP as described below. Scaling up is usually not necessary but if a higher yield of immunoprecipitated DNA is required, we recommend performing separate ChIPs and pool the immunoprecipitated DNA before purification.

For using lower amount of tissue per IP:

- You can start with 30-40 mg of tissue and follow the protocol up to the chromatin shearing step. Then simply dilute the sheared chromatin in shearing buffer S1 before adding it to the IP reaction. The final volume of diluted chromatin containing the desired starting amount should be 100 µl per IP reaction.

- You can start with a smaller batch of tissue. First, determine the amount that you will use per IP and the total number of IPs. Follow the standard protocol for tissue fixation, collection and lysis. Do not scale lysis buffers L1b and L2. Define the volume of shearing buffer S1 taking into account that you will need:

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

• 1 µl of sheared chromatin per input

• 50 µl of sheared chromatin for chromatin shearing assessment

• Add 5% excess of S1

Resuspend the tissue in the required volume of shearing buffer S1 and follow the standard protocol.

Please note that changing 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 required per IP is 0.3-3 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 additional optimization but usually a fixation of 8 to 10 mins is suitable for most histone proteins. Please note that a longer fixation may lead to chromatin that is resistant to sonication.

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 series of ChIP reactions.

Positive and Negative 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. We also recommend using the positive control ChIP-seq grade H3K4me3 antibody at least once.

Quantification

After the ChIP, determine the concentration of the immunoprecipitated DNA with a highly sensitive method such as the dsDNA HS Assay Kit on the Qubit® system from Thermo Fisher 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 immunoprecipitated 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 H3K4me3 antibody on 1,000,000 HeLa cells is approximately 10 ng.

Quantitative PCR analysis

Prior to the sequencing, we recommend analyzing 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 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.

Technical Support

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