Version 1a Last updated 28 June 2021

Pre-ChIP Assay Preparation Guidelines for ChIP Kit (Magnetic, qPCR) ab270816

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ChIP Kit (Magnetic, qPCR) datasheet:

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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

The protocol describes the preparation of a batch of chromatin which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. The starting number of cells per batch depends on the target to be studied: histone marks require less starting material than transcriptional factors. Depending on the target we recommend to work with the following numbers of cells:

Target	Cells/batch	Cells/IP	No. of IPs
Transcription factors	25,000,000	~4,000,000	6 IPs + 1 INPUT + 1 shearing assessment
Histones	7,000,000	1,000,000	6 IPs + 1 INPUT + 1 shearing assessment

Scale the amount of cells per batch accordingly to the experimental plan.

Δ **Note:** The described protocol uses a prompt direct fixation in the cell culture plate. Fixed and scraped cells can not 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 number of cells available, it may be possible to scale up and down the number of cells per IP and/or start with a smaller or a bigger batch of cells.

For using lower numbers of cells per IP, you can start with a recommended batch 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 L2 per 1 million cells. Define the volume of Shearing Buffer S1b taking into account that you will need:

	Volume
Sheared chromatin (containing the desired amount of cells) per IP reaction	250 µl
Sheared chromatin per input	2.5 µl
Sheared chromatin for chromatin shearing assessment	50 µl
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.

If you are studying both histones and transcription factors from the same batch, please, refer to our recommendations for TFs. Then for histone marks simply dilute the sample(s) in Shearing Buffer S1b to obtain 1,000,000 cells per IP.

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.

Tissue Amount

The protocol describes the preparation of a batch of chromatin which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. The starting amount of tissue per batch depends on a target to be studied: histone marks require less starting material than transcriptional factors. Depending on the target we recommend to work with the following amounts of cells:

Target	Tissue/batch	Tissue/IP	No. of IPs
Transcription factors	200 mg	~30 mg	6 IPs + 1 INPUT + 1 shearing assessment
Histones	40 mg	~7 mg	6 IPs + 1 INPUT + 1 shearing assessment

Scale the amount of tissue per batch accordingly to the experimental plan.

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 or scale 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 a recommended amount of tissue 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 a starting higher amount of tissue per IP is desired, first determine the amount of tissue that you will use per IP and the total number of IPs.

Fix the tissue 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 S1b taking into account that you will need:

	Volume
Sheared chromatin (containing the desired amount of tissue) per IP reaction	250 µl
Sheared chromatin per input	2.5 µl
Sheared chromatin for chromatin shearing assessment	50 µl
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. The amount of chromatin per IP for histone marks should be in the range 0.5-3 µg, while a higher amount (3-10 µg) is required for transcription factors. We recommend performing a pilot experiment to determine the optimal amount of tissue. Once determined, it should be kept constant between experiments.

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.

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. Generally, a shorter fixation is required for histone marks (8-10 min) than for transcription factors (10-20 min) using a standard formaldehyde single step fixation protocol.

Δ Note: A longer fixation times 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 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. 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 DiaMag 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.

Negative Control

The kit contains a negative IgG antibody. We recommend including one negative IgG control in each series of ChIP reactions.

Quantitative PCR analysis

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. 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 although performing in triplicate is recommended to be able to identify potential outliers.

Technical Support

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