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ab270816 ChIP Kit (Magnetic, qPCR)

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ChIP Kit (Magnetic, qPCR) datasheet:

www.abcam.com/ab270816

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

Table of Contents

1. Overview	1
2. Materials Supplied and Storage	2
3. Materials Required, Not Supplied	3
4. General guidelines, precautions, and troubleshooting	4
5. Protocol	5
6. Notes	16

1. Overview

ChIP Kit (Magnetic, qPCR) (ab270816) is a highly optimized and validated solution for ChIP-qPCR assays for either histones or transcription factors. The complete kit contains everything you need for start-to-finish ChIP including all validated buffers and reagents for chromatin shearing, immunoprecipitation, and DNA isolation for exceptional ChIP-qPCR results.

This kit uses a unique and fast method for DNA isolation and decrosslinking within 30 minutes compared to the standard 4 hours. Overall, easy to use and rapid protocol allows to receive the results within 20 hours with 4 hours hands-on time.

Sample size: Histones -

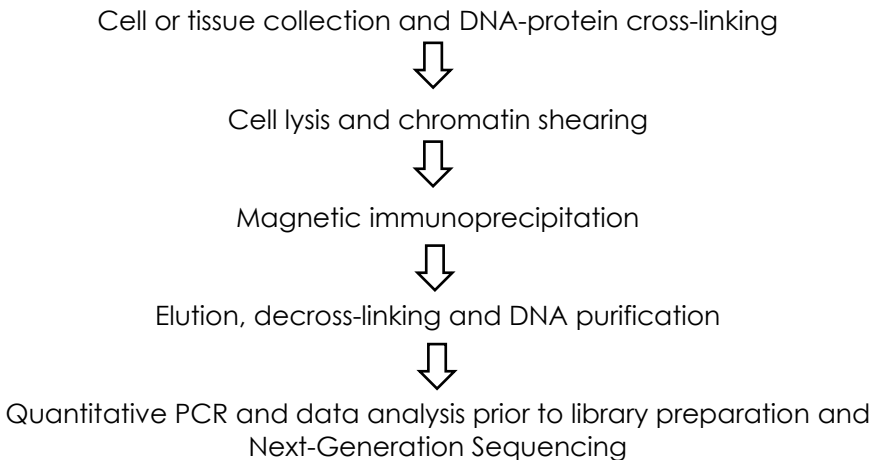
Cells (1,000,000 cells per IP)

Tissues (7 mg of tissue per IP)

Sample size: Transcription factors -

Cells (4,000,000 cells per IP)

Tissues (30 mg of tissue per IP)



2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. Please see table below for individual storage conditions.

Component	Quantity 24 tests	Storage
Protease inhibitor cocktail	80 µl	-20°C
5% BSA (DNA free)	784 µl	-20°C
Proteinase K	32 µl	-20°C
Rabbit IgG	8 µg	-20°C
Glycine	8.8 ml	4°C
Shearing Buffer S1b	9 ml	4°C
Protein A-coated magnetic beads	720 µl	4°C (do not freeze)
Wash Buffer W1	8.4 ml	4°C
Wash Buffer W2	8.4 ml	4°C
Wash Buffer W3	8.4 ml	4°C
Wash Buffer W4	8.4 ml	4°C
ChIP-seq grade water	26.6 ml	4°C
Fixation Buffer	8 ml	4°C
DNA isolation Buffer (DIB)	3.2 ml	4°C
5x ChIP Buffer C1b	6.9 ml	4°C
Lysis Buffer L1b	100 ml	4°C
Lysis Buffer L2	60 ml	4°C
Elution Buffer E1	220 µl	4°C
Elution Buffer E2	20 µl	4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Gloves
- RNase/DNase-free 1.5 ml and 15 ml tubes
- Formaldehyde, 37%, molecular grade
- Phosphate buffered saline (PBS) buffer
- Trypsin-EDTA
- Cell culture medium
- qPCR SYBR® Green Mastermix
- ChIP-seq grade antibodies
- Fluorescence-based assay for DNA concentration measurement
- Dounce homogenizer with loose a pestle (2 ml)
- 1.5ml magnetic rack
- Scalpel
- Petri dishes
- Cell counter system
- Sonication device and the associated microtubes
- Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
- Rotating wheel
- Vortex
- Thermomixer
- Fluorometer
- qPCR cycler

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Protocol

- [Before proceeding please read Pre-ChIP Assay Preparation Guidelines for this kit on the product datasheet](#)
- Protocol for the first step differs for cells (step 5.1) and tissues (step 5.3).

5.1 Cell collection and DNA-protein cross-linking from cultured cells.

- 5.1.1 Equilibrate the Fixation Buffer to room temperature before use.
- 5.1.2 Prepare the cross-linking solution in a fume hood by adding formaldehyde to the Fixation Buffer to a final concentration of 11% (e.g. add 0.6 ml of 37% formaldehyde to 1.4 ml of Fixation Buffer). Add the diluted formaldehyde directly to the cell culture medium in a proportion of 1:10. For a 20 ml cell culture you will need 2 ml of cross-linking solution.
- 5.1.3 Incubate the cells for 10 minutes at room temperature with gentle shaking.
Δ Note: The fixation time might require an additional optimization.
- 5.1.4 Add Glycine to the cell culture medium in a proportion of 1:10 to stop the fixation (e.g. add 2.2 ml of glycine when you started with a 20 ml cell culture volume). Incubate for 5 minutes at room temperature with gentle shaking. Proceed to the next step immediately.
Δ 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 4 months. To freeze the cells, remove the medium, wash the cells once with 20 ml of PBS. Add another 5 ml of PBS, collect the cells by scrapping and/or centrifugation at 500 *x g* for 5 minutes and 4°C and discard the supernatant. Store the cell pellet at -80°C.

5.2 Cell lysis and chromatin shearing for cultured cells.

For adherent cells:

- 5.2.1 Remove the medium and wash the cells once with 20 ml of PBS. Discard the PBS. Keep everything at 4°C or on ice from now on.
- 5.2.2 Add 5 ml of cold Lysis buffer L1b to the plate. Collect the cells by scraping and transfer them into a 50 ml tube.
- 5.2.3 Rinse the flask with the amount of Lysis Buffer L1b recommended in the below table and add this to the 50 ml tube.

	Buffer L1b
TF (25,000,000 cells)	20 ml
Histones (7,000,000 cells)	2 ml

Δ Note: For up or down scaling use 1 ml of buffer L1b per million of cells.

- 5.2.4 Proceed immediately with step 5.2.5.

For suspension cells:

- 5.2.5 Transfer cells into a 50 ml tube. Pellet the cells by centrifugation at 500 *x g* and 4°C for 5 minutes. Discard the cell culture medium.
- 5.2.6 Resuspend the cells in 20 ml of ice-cold PBS, centrifuge at 500 *x g* and 4°C for 5 minutes and gently discard the supernatant. Keep everything at 4°C or on ice from now on.
- 5.2.7 Resuspend the cells in 1 ml of ice-cold Lysis Buffer L1b by pipetting up and down several times. Add the amount of buffer L1b from the table below:

	Buffer L1b
TF (25,000,000 cells)	24 ml
Histones (7,000,000 cells)	6 ml

Δ Note: For up or down scaling use 1 ml of L1b per 1 million cells.

- 5.2.8 Proceed immediately with step 5.2.9.

- 5.2.9 Incubate at 4°C for 20 minutes with gentle mixing on a rotator. Pellet the cells by centrifugation at 500 $\times g$ and 4°C for 5 minutes and discard the supernatant.
- 5.2.10 Resuspend the cell pellet in 1 ml of ice-cold Lysis Buffer L2 by pipetting up and down several times. Add the amount of buffer L2 from the below table:

	Buffer L1b
TF (25,000,000 cells)	14 ml
Histones (7,000,000 cells)	3.2 ml

Δ Note: For scaling up or down, use 600 μ l of buffer L2 per 1 million of cells.

- 5.2.11 Incubate for 10 minutes at 4°C with gentle mixing on a rotator. Pellet the cells again by centrifugation for 5 minutes at 500 $\times g$ and 4°C and discard the supernatant.
- 5.2.12 Add 8.4 μ l of 200x protease inhibitor cocktail to 1.67 ml of Shearing Buffer S1b. Keep on ice.
- 5.2.13 Add the complete Shearing Buffer S1b to the cell pellet. The cell concentration in the Shearing Buffer should be as following:

TF	1.5 million cells per 100 μ l S1b
Histones	420,000 cells per 100 μ l S1b

- 5.2.14 Resuspend the cells by pipetting up and down several times and incubate on ice for 10 minutes. Split the cell suspension into aliquots of maximum 300 μ l by transferring it to the appropriate sonication microtubes.
- Δ Note:** The use of correct type of microtubes is essential for the efficiency of sonication.
- 5.2.15 Shear the chromatin by sonication using the Bioruptor. Choose the protocol which is adapted to your device. For example, when using the Bioruptor Standard or Plus, shear for 10 to 30 cycles [30 seconds "ON", 30 seconds "OFF"] each at High power setting. Or, when using the Bioruptor Pico shear for 5 to 12 cycles [30 seconds "ON", 30 seconds "OFF"].
- Δ Note:** We recommend performing pilot experiments for each new sample type.
- 5.2.16 Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at

- 16,000 $\times g$ and 4°C for 10 minutes. Pool the supernatants which contain the sheared chromatin.
- 5.2.17 Take an aliquot of 50 μ l of the sheared chromatin for shearing assessment as described in a separate protocol.
- Δ Note:** We recommend analyzing the shearing for each batch of chromatin. This step can be omitted when optimal sonication settings for given experimental conditions (cell type, cell number and fixation) have been established previously.
- 5.2.18 Use the chromatin for magnetic immunoprecipitation or store it at -80°C for up to 2 months.

5.3 Tissue disaggregation and DNA-protein cross-linking.

- 5.3.1 Equilibrate the Fixation Buffer to room temperature before use.
- 5.3.2 Prepare the cross-linking solution in a fume hood by adding 54 μ l of 37% formaldehyde to 2 ml of Fixation Buffer to a final concentration of 1%. Use 2 ml of Fixation Buffer for one chromatin preparation.
- 5.3.3 Put the required amount of fresh or frozen tissue (up to 200 mg) in a petri dish on ice. Keep samples on ice at all times and minimize the time of manipulation to prevent sample degradation.
- 5.3.4 Chop the tissue into small pieces (between 1-3 mm³) using a scalpel blade and transfer it into a Dounce homogenizer. Add 1 ml of formaldehyde diluted in Fixation Buffer.
- 5.3.5 Immediately disaggregate the tissue using a Dounce homogenizer (loose fitting pestle) to get a homogeneous suspension. Set the timer for a total fixation time of 10 minutes. Start deducting the fixation time from this point.
- Δ Note:** The fixation time might require an additional optimization.
- 5.3.6 Transfer the tissue suspension into a 15 ml tube. Rinse the Dounce homogenizer with an additional 1 ml of diluted formaldehyde and pool with the sample in the same 15 ml tube.
- 5.3.7 Incubate for a total fixation time of 10 minutes (starting at step 5.3.5) at room temperature with gentle rotation on a rotator.
- 5.3.8 Add 200 μ l of Glycine to the tissue suspension to stop the fixation. Incubate for 5 minutes at room temperature with gentle mixing on a rotator. Proceed to the next step immediately.

Δ Note: We strongly recommend using freshly fixed tissue for preparation of sheared chromatin prior to ChIP. If not possible, the fixed tissue can be stored at -80°C for up to 4 months. To freeze the tissue, remove the medium and wash the tissue once with 2 ml of PBS. Store the tissue pellet at -80°C.

5.4 Cell lysis and chromatin shearing for tissue samples.

- An additional 100 µl of protease inhibitors cocktail is required per chromatin preparation – not supplied with this kit.
- 5.4.1 Centrifuge samples at 850 $\times g$ for 5 minutes at 4°C. Gently discard the supernatant and keep the pellet.
- 5.4.2 Wash the pellet with 10 ml of ice-cold PBS and centrifuge samples at 850 $\times g$ for 5 minutes at 4°C. Gently discard the supernatant. Keep everything at 4°C or on ice from now on.
- 5.4.3 Add 50 µl of 200x protease inhibitor cocktail to 10 ml of ice-cold Lysis Buffer L1b.
- 5.4.4 Add 1 ml of ice-cold complete Lysis Buffer L1b to the pellet and resuspend by pipetting up and down several times. Add the remaining amount of complete Buffer L1b.
- 5.4.5 Incubate at 4°C for 20 minutes with gentle mixing on a rotator.
- 5.4.6 Pellet the cells by centrifugation at 850 $\times g$ for 5 minutes at 4°C and discard the supernatant.
- 5.4.7 Add 50 µl of 200x protease inhibitor cocktail to 10 ml of ice-cold Lysis Buffer L2. Add 1 ml of ice-cold complete Lysis Buffer L2 to the cell pellet and resuspend the cells by pipetting up and down several times. Add the remaining amount of complete Buffer L2.
- 5.4.8 Incubate at 4°C with gentle mixing on a rotator for 10 minutes.
- 5.4.9 Pellet the cells again by centrifugation at 850 $\times g$ for 5 minutes at 4°C and discard supernatant.
- 5.4.10 Add 8.4 µl of 200x protease inhibitor cocktail to 1.67 ml of Shearing Buffer S1b. Keep on ice.
- 5.4.11 Add the complete Shearing Buffer S1b to the pellet. Resuspend the cells by pipetting up and down several times and homogenize using a bounce homogenizer (tight pestle). Incubate for 10 minutes on ice.

- 5.4.12 Split the cell suspension into aliquots of maximum 300 μ l by transferring it to the appropriate 1.5 ml microtubes.
Δ Note: The use of correct type of microtubes is essential for the efficiency of sonication.
Shear the chromatin by sonication.
Δ Note: We recommend performing pilot experiments for each new sample type.
- 5.4.13 Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 $\times g$ at 4°C for 10 minutes. Pool the supernatants which contain the sheared chromatin.
- 5.4.14 Optional step: DNA concentration in the supernatant can be quantified at this step. We recommend using fluorometric quantitation (eg. Qubit assay).
- 5.4.15 Take an aliquot of 50 μ l of sheared chromatin for the shearing assessment. The protocol for chromatin shearing analysis is described in a separate protocol.
Δ Note: We recommend analyzing the shearing for each batch of chromatin. This step can be omitted when optimal sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimized previously.
- 5.4.16 Use the chromatin in magnetic immunoprecipitation or store it at -80°C for up to 2 months.

5.5 Magnetic immunoprecipitation for cells and tissues.

5.5.1 Determine the amount of IP reactions to perform including the negative control IP (IgG). Take 30 μ l of Protein A-coated magnetic beads per IP and transfer it to a clean 1.5 ml tube.

5.5.2 Prepare 4 ml of 1x ChIP Buffer C1b by mixing the following reagents:

- 3.2 ml ChIP-seq grade water
- 0.8 ml 5x ChIP Buffer C1b
- 80 μ l of 5% BSA

Keep the diluted ChIP Buffer C1b on ice.

Δ Note: 4 ml of 1x ChIP Buffer C1b is enough to wash the beads for up to 6 IPs

5.5.3 Wash the beads 3 times with 1 ml of ice-cold 1x ChIP Buffer C1b. To wash the beads, add 1 ml of 1x ChIP Buffer C1b directly to the bead suspension, resuspend the beads by pipetting up and down several times and incubate at 4°C with gentle shaking for 5 minutes. Spin the tubes and place them in the 1.5 ml magnetic rack. Wait for one minute to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 2 times.

5.5.4 After the last wash, resuspend the beads in 1x ChIP Buffer C1b by adding the original volume of beads (30 μ l per IP).

5.5.5 Take the required number of 1.5 ml tubes (one tube per IP) and add 30 μ l of the washed beads to each tube.

5.5.6 Prepare the ChIP reaction mix for 1 IP as described below. Scale according to the number of IPs including a small excess (0.5 extra reaction).

Δ Note: Prepare separate reaction mixes if you use different antibodies or different amounts of antibody.

- 6 μ l of BSA
- 1.8 μ l 200x protease inhibitor cocktail
- 20 μ l 5x C1b Buffer
- (42.2 μ l – x μ l) water
- Add x μ l of ChIP-grade antibody

The total volume of the ChIP reaction mix per IP is 70 μ l.

Δ Note: The required amount of antibody per IP may vary. Check the supplier's recommendation or perform a titration curve using different amounts of antibody. Use 1 μ g of IgG (negative control antibody) for the negative control IP. If required, NaBu (20 mM final concentration) or other inhibitors can be added.

- 5.5.7 Add 70 μ l of ChIP reaction mix to the individual tubes containing 30 μ l of washed Protein A-coated magnetic beads. Incubate the tubes for 2-4 hours at 4°C under constant rotation on the rotator.
- 5.5.8 Briefly spin the tubes containing the ChIP reaction mix and add 250 μ l of sheared chromatin. Keep aside 2.5 μ l of the sheared chromatin at 4°C to be used as an INPUT. Incubate the tubes overnight at 4°C under constant rotation on the rotator.
- 5.5.9 Perform the washes as follows: briefly spin the tubes and place them in the magnetic rack. Wait for 1 minute and remove the supernatant. Add 350 μ l of Wash Buffer W1: gently shake the tubes to resuspend the beads and incubate for 5 minutes on the rotator at 4°C.
- 5.5.10 Repeat the washing step as described above once with Wash Buffer W2, W3 and W4, respectively.

5.6 DNA isolation and decross-linking for cells and tissues.

- 5.6.1 Prepare complete DIB Buffer by adding 1 μ l of Proteinase K to 100 μ l of DIB Buffer. Scale according to the number of samples including the input sample (you will need 100 μ l per IP sample and 97.5 μ l for the input).
- 5.6.2 Briefly spin the tubes containing the IP samples and place them in the magnetic rack. Wait for 1 minute and remove the supernatant.
- 5.6.3 Briefly centrifuge the input sample and treat it in parallel with the IP samples from this point onwards.
- 5.6.4 Remove the tubes from the magnetic rack and add 100 μ l of complete DIB to the IP samples. Resuspend the beads by pipetting up and down a few times. Add 97.5 μ l complete DIB to the input sample.
- 5.6.5 Incubate at 55°C for 15 minutes followed by 15 minutes at 100°C.
- 5.6.6 Briefly spin down the tubes to bring down the liquid caught in the lid.
- 5.6.7 Place the tubes in the magnetic rack and wait for 1 minute.
- 5.6.8 Transfer the supernatant which contains the DNA to new labeled tubes. Determine the total number of regions to be analyzed by qPCR for each sample and take the required volume for qPCR analysis.
Δ Note: Depending on a sensitivity of a Master Mix and qPCR cyclers used the samples can be diluted before PCR and the volume per PCR may vary
- 5.6.9 Store the remaining DNA at -20°C until further use.

5.7 Quantitative PCR analysis for cells and tissues.

- For each primer pair, run the Input DNA alongside the immunoprecipitated samples and negative IgG control.
- 5.7.1 Take an aliquot of immunoprecipitated DNA and a corresponding INPUT.
- 5.7.2 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

Use the following PCR program:

Step	Time/cycles	Temperature	
Denaturation	3-10 mins*	95°C	
Amplification	30 secs	40 cycles	95°C
	30 secs		60°C
	30 secs		72°C (acquire fluorescent data)
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

Δ Note: These conditions may require optimization depending on the type of master mix, qPCR system used and user provided primer pair.

- 5.7.3 Record the threshold cycles (Ct values) from the exponential phase of the qPCR for the immunoprecipitated DNA sample and input for each primer pair.
- 5.7.4 Calculate the relative amount of immunoprecipitated DNA compared to INPUT DNA for the control regions (% of recovery) using the following formula:

$$\% \text{ recovery} = 2^{((Ct_{\text{input}} - 6.64) - Ct_{\text{sample}})} \times 100\%$$

- Ctsample and Ctinput are the threshold cycles from the exponential phase of the qPCR for the IP'd DNA sample and INPUT, respectively.

- 2 is the amplification efficiency

- 6.64 is a compensatory factor to correct the input dilution

Δ 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 the amount of INPUT used is different from 1%, an introduction of a compensatory factor in the formula is required to correct the input dilution (x) as follows:

$$\% \text{recovery} = 2^{[(Ct_{\text{input}} - \log_2(X\%)) - Ct_{\text{sample}}]} \times 100\%$$

Where: $\log_2(x)$ accounts for the INPUT dilution

Example: If you use an INPUT of 5 μ l from 250 μ l of chromatin used per IP, it corresponds to 50 X dilution. The compensatory factor is equal to $\log_2(50)=5.64$ and the formula to calculate the recovery will be as follows:

$$\% \text{recovery} = 2^{[(Ct_{\text{input}} - 5.64) - Ct_{\text{sample}}]} \times 100\%$$

6. Notes

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