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ab270814 ChIP Kit (low cell number)

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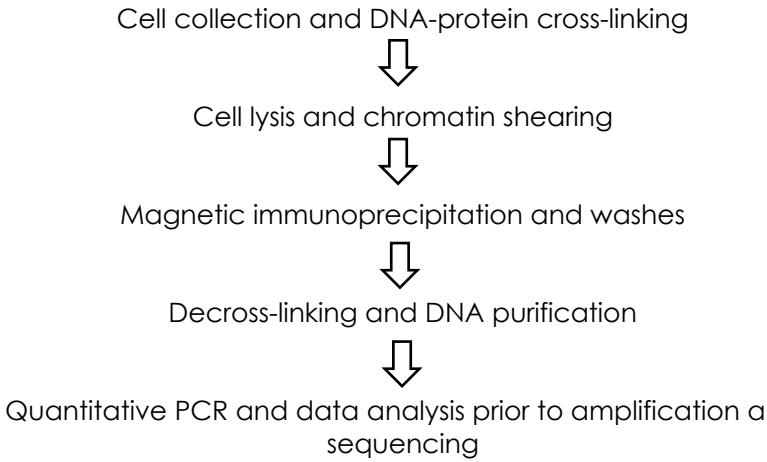
1. Overview

ChIP Kit (low cell number) (ab270814) contains everything you need for start-to-finish ChIP including all validated buffers and reagents for chromatin shearing, immunoprecipitation, and elution for exceptional ChIP-qPCR or ChIP-Seq results. In addition, positive control antibodies and negative control PCR primers are included for your convenience and assurance of result sensitivity and specificity.

Sample size:

Cells (10,000 cells per IP)



2. Materials Supplied and Storage

Component	Quantity 16 tests	Storage
Bead Wash Buffer BW1	13.6 ml	4°C
ChIP Buffer C1	4.5 ml	4°C
ChIP-seq grade antibody H3K4me3	8 µg	-20°C
ChIP-seq grade GAPDH TSS primer pair	40 µl	-20°C
ChIP-seq grade Myoglobin exon 2 primer pair	40 µl	-20°C
Co-precipitant CP1	50 µl	-20°C
Co-precipitant CP2	50 µl	-20°C
DiaMag protein A-coated magnetic beads	180 µl	4°C*
Elution Buffer E1	11 ml	4°C
Elution Buffer E2	410 µl	4°C
Glycine	4.5 ml	4°C
Lysis Buffer L1	1 ml	4°C
Precipitant P1	1 ml	4°C
Protease inhibitor cocktail 200x (PIC)	225 µl	-20°C
Rabbit IgG	8 µg	-20°C
Wash Buffer W1	4.5 ml	4°C
Wash Buffer W2	4.5 ml	4°C
Wash Buffer W3	4.5 ml	4°C
Wash Buffer W4	4.5 ml	4°C

*Do NOT freeze magnetic beads because they may become damaged.
Keep the beads in liquid suspension during storage as drying will result in reduced performance.

3. Materials Required, Not Supplied

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

- Spin columns
- Gloves to wear at all steps
- Phosphate buffered saline (PBS)
- Cell culture medium
- 1 M Sodium butyrate (NaBu) (optional)
- Trypsin-EDTA
- Formaldehyde (fresh MolBiol Grade)
- Hank's balanced salt solution (HBSS)
- Ethanol
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- qPCR reagents
- Quant-IT dsDNA HS assay kit or equivalent
- TE
- 1.5ml magnetic rack
- Refrigerated centrifuge for 1.5 ml tubes
- Cell counter
- Eppendorf Low retention 1.5 ml Tubes
- Sonication apparatus
- 1.5 ml TPX microtubes (optimized for chromatin shearing with sonicator)
- Rotator (rotating wheel)
- Thermomixer (65°C)
- Vortex
- Qubit system
- 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 the Pre-Chip Assay Preparation Guidlines and General Protocol for Chromatin Shearing Analysis documents on the product datasheet

- The protocol below is for use with 10,000 cells per ChIP.

5.1 Cell collection and DNA-protein cross-linking.

5.1.1 Prepare and harvest cells as follows:
- Place PBS, cell culture medium and trypsin-EDTA at RT.
- If using adherent cells, discard medium to remove dead cells. Wash cells by adding 10 ml PBS. Detach cells by trypsinization. Collect cells by adding culture medium and transfer the medium with cells in a 15 ml centrifugation tube. Use culture medium containing serum (you can the same medium as the one used for culturing the cells). Centrifuge 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant.
- If using suspension cells, centrifuge for 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant.

5.1.2 Resuspend the cells in cell culture medium. You should have at least 10,000 cells per ml of cell culture medium. Count the cells.

5.1.3 Label new 1.5 ml tube(s). Add medium to a final volume of 1 ml after the cells have been added. To determine the amount of cells to use for fixation, determine the number of

immunoprecipitations you will perform and start fixation of a unique batch of chromatin.

5.1.4 Add 27 µl of 36.5% formaldehyde per 1 ml of sample (final concentration should be ~1%) and invert tubes immediately two to three times to ensure complete mixing.

5.1.5 Incubate for 10 minutes at room temperature to enable fixation with occasional manual agitation. Optimization of fixation time may be required depending cell type, it could be 8-10 minutes.

5.1.6 Add 115 µl of Glycine to the sample.

5.1.7 Mix by inversion of the tube four to five times. Incubate for 5 minutes at room temperature to stop the fixation. Work on ice from this point onwards.

5.1.8 Centrifuge at 300 *x g* for 10 minutes at 4°C.
Δ Note: We recommend the use of a swing-out rotor with soft settings for deceleration.

5.1.9 Aspirate the supernatant slowly and leave approximately 30 µl of the solution. Do not disturb the pellet. Take care to not remove these cross-linked cells.

5.1.10 Wash the cross-linked cells with 1 ml of ice cold HBSS containing protease inhibitor cocktail (PIC, 200x; final concentration 1x).

- Add 1 ml of HBSS and invert the tube four to five times to resuspend the cells.
- When working with higher cell numbers (100,000 cells and more) you should gently vortex to completely resuspend the cells.
- Centrifuge at 300 *x g* for 10 minutes at 4°C (in a swing-out rotor with soft settings for deceleration).

5.1.11 Discard the supernatant and keep the cell pellet on ice. Proceed directly to cell lysis or if desired the cell pellets can be store at -80°C for up to 2 months.

5.2 Cell lysis and chromatin shearing.

5.2.1 Prepare Lysis Buffer. Add protease inhibitor cocktail (1x final concentration) to Lysis Buffer L1 (RT). This is the complete Lysis Buffer L1. Keep the buffer at room temperature until use. Discard what is not used within a day.
Δ Note: Make sure that there are no crystals in the Lysis Buffer L1 before using. Gently heat and mix until crystals disappear.

5.2.2 Add complete Lysis Buffer L1 to the cells. Use 25 µl of complete Lysis Buffer L1 per 10 000 cells. Agitate manually the bottom of the tube to resuspend the cells and allow bubbles to form. Scale accordingly when using higher numbers of cells.

5.2.3 Incubate on ice for 5 minutes to ensure complete cell lysis.

5.2.4 Add HBSS containing protease inhibitor cocktail (PIC, 200x; final concentration 1x) to the cell lysate. Use 75 µl of complete HBSS per 10,000 cells. Scale accordingly when using higher numbers of cells.

5.2.5 Dispense 100 µl of cell lysate (equivalent to 10,000 cells) into 1.5 ml microtubes. If cell lysis was performed on more than 10,000 cells, make sure that there is no precipitate before splitting cell lysate into 100 µl aliquots. Otherwise gently heat until crystals disappear.

5.2.6 Sonicate samples to shear the chromatin using 1-5 runs of 5 cycles of: [30 seconds "ON", 30 seconds "OFF"] each. Optimization may be required depending on cell type and density.

- We recommend using a water cooler to maintain the temperature around 4°C during shearing. In brief: pre-cool the bath with ice, and then remove ice. Then add 4°C water and crushed ice to water level mark. Set the sonicator to "High Power". Replace water with 4°C water and ice every 5 cycles to maintain the temperature below 8°C. Place samples on ice while changing the water.

5.2.7 Centrifuge at 14,000 *x g* (13,000 rpm) for 10 minutes and collect the supernatant which contains the sheared chromatin. Analysis of shearing efficiency is recommended before starting a ChIP, especially when a particular cell type is used for the first time.

5.2.8 Use the sheared chromatin directly in ChIP. However, if desired the chromatin can be store at -80°C for up to 2 months.

5.3 Magnetic immunoprecipitation and washes.

5.3.1 Prepare complete ChIP Buffer C1. Add protease inhibitor cocktail (PIC, 200x; final concentration 1x) to ChIP Buffer C1. This is complete ChIP Buffer C1.

5.3.2 Add 100 µl of complete ChIP Buffer C1 per 100 µl of sheared chromatin.

5.3.3 Use 200 µl of diluted sheared chromatin per tube for each IP. Use low retention 1.5 ml tube. Set aside 20 µl as input sample and keep at 4°C.

5.3.4 Add the specific antibody or control antibodies (positive and negative) to each tube. We recommend including one IgG negative control in each series of ChIP reactions.

5.3.5 Incubate the IP tubes at 40 rpm on a rotating wheel for 16 hours at 4°C.

5.3.6 Prepare magnetic beads. Each IP requires 11 µl of beads. Add 55 µl Beads Wash Buffer BW1 to 11 µl stock solution of beads for each IP and scale accordingly. Resuspend the beads and place them in the magnetic rack. Discard the supernatant and keep the beads captured. Repeat this wash once. Then resuspend the bead pellet in 11 µl of Beads Wash Buffer BW1 per IP reaction.

5.3.7 Add 10 µl of pre-washed Protein A-coated beads to each IP tube.

5.3.8 Incubate the IP tubes at 40 rpm on a rotating wheel for 2 hours at 4°C.

5.3.9 Briefly spin the tubes and place them in the magnetic rack. Wait for one minute and remove the supernatant. Wash the beads with Wash Buffer W1. To wash the beads, add 100 µl of Wash Buffer W1, gently shake the tubes to resuspend the beads and incubate for 4 minutes on a rotating wheel (40 rpm) at 4°C.

5.3.10 Repeat the wash as described above once with Wash Buffer W2, W3 and W4, respectively.

5.4 DNA decross-linking and purification.

5.4.1 After removing the last Wash Buffer, add 200 µl of Elution Buffer E1 to the beads and incubate for 30 minutes on a rotating wheel at room temperature. Also add 180 µl of Elution Buffer E1 to 20 µl of the input sample kept aside the day before.

5.4.2 Briefly spin the tubes and place them in the magnetic rack. Transfer the supernatant to a new tube and add 8 µl of Elution Buffer E2. Also add 8 µl Elution Buffer E2 to the input sample. Incubate for 4 hours in a thermomixer at 1300 rpm and 65°C.

5.4.3 DNA purification using spin columns.

- In a 1.5 ml microcentrifuge tube, add 5 volumes of ChIP DNA Binding buffer to each volume of sample (1040 µl: 208 µl). Mix briefly.
- Transfer mixture to a provided Spin column in a Collection tube.
- Centrifuge at ≥ 10,000 *x g* for 30 seconds. Discard the flow-through.
- Add 200 µl DNA Wash buffer to the column. Centrifuge at ≥ 10,000 *x g* for 30 seconds. Repeat wash step.
- Add 6-100 µl DNA Elution buffer directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥ 10,000 *x g* for 30 seconds to elute the DNA.

5.5 Quantitative PCR analysis and data analysis.

- Before sequencing the samples, we recommend analyzing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. In order to have sufficient DNA left for quantification and sequencing, we recommend using one third of the total immunoprecipitated DNA for qPCR analysis.

5.5.1 Prepare the qPCR mix using SYBR Green master mix. qPCR mix (total volume of 25 µl/reaction):

- 1 µl of primer pair (stock: 5 µM each: reverse and forward)
- 12.5 µl of master mix (e.g.: iQ SYBR Green supermix)
- 5.0 µl of purified diluted DNA sample and purified input(s)
- 6.5 µl of water

5.5.2 Use the following PCR program: 3 to 10 minutes denaturation step at 95°C (please check carefully supplier's recommendations about Taq polymerase activation time), followed by 45 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72°C. These conditions may require optimization depending on the type of Master Mix or qPCR system used.

6. Notes

Technical Support

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