ChIP Elute Kit Protocol-At-A-Glance

The **ChIP Elute Kit** (Cat. No. 634887) is a fast and very simple tool to replace the slow and tedious DNA recovery steps commonly performed at the end of chromatin immunoprecipitation (ChIP) experiments. Typical ChIP experiments involve DNA–protein cross-linking, chromatin isolation and fragmentation, and finally immunoprecipitation of targeted DNA-bound proteins, thus resulting in DNA–protein complexes bound to a solid support through an antibody bridge. Before downstream experiments can be performed, fragmented DNA must be eluted from the beads, dissociated from accompanying nuclear proteins, and then purified. The ChIP Elute Kit combines the DNA elution and protein dissociation (cross-linking reversal) into a single, simultaneous step. The DNA is then purified and concentrated with ChIP-grade reagents included in the kit. The resulting single-stranded DNA (ssDNA) is compatible with qPCR applications or direct sequencing library preparation using the DNA SMART™ ChIP-Seq Kit, which does not require double-stranded DNA (dsDNA) input. The ChIP Elute Kit is compatible with any upstream ChIP protocol, and efficiently isolates, purifies, and concentrates ChIP DNA in less than one hour.

I. List of Components

See the ChIP Elute Kit Certificate of Analysis for more information about storage conditions and technical specifications.

ChIP Elute Kit (Cat. No. 634887; 50 rxns)	
Package 1 (Store at 4°C.)	
ChIP Elute Resin	2 x 3 ml
Proteinase K	100 µl
DNA Dilution Buffer	10 ml
Package 2 (Store at room temperature.)	
ssDNA Binding Columns	50 columns
Collection Tubes	50 tubes
ssDNA Binding Buffer	25 ml
ssDNA Wash Buffer	25 ml

NOTE: Before the first use, add 100 ml of 100% ethanol to the ssDNA Wash Buffer.

II. Additional Materials Required

The following reagents are required but not supplied:

- Pipettes and filter tips
- 1.5 ml low-retention tubes such as Eppendorf, Cat. No. 022431021
- Two heat blocks set to 95°C and 55°C
- Centrifuge that accommodates 1.5 ml tubes
- TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- 100% ethanol (added to the ssDNA Wash Buffer before the first use)

III. Protocol: DNA elution and cross-linking reversal

The input material for this kit is Protein A/G agarose (or magnetic) beads, and the associated captured nuclear protein–DNA complexes, from a ChIP assay (in low-retention tubes). Prepare your samples following your usual ChIP protocol, including appropriate detergent/salt washes, but **not** DNA elution and cross-linking reversal steps.

This kit was designed to be used on native or formaldehyde cross-linked chromatin. Other cross-linking agents have not been tested.

DNA tends to stick to plastic at low concentrations and during freeze-thaw cycles. The use of low-retention tubes helps to minimize this sticking, which reduces DNA losses. We strongly recommend you exclusively use low-retention tubes throughout this protocol.

1. **If your ChIP protocol already contains TE washes move on to step 2.** After the detergent/salt washes from your ChIP protocol, wash the protein/DNA/bead complexes using TE buffer. Collect beads at the bottom of the tube, remove any remaining liquid, and add 1 ml TE buffer. Resuspend the beads by rotating the tube for 2 min at room temperature. Then briefly spin to collect the beads at the bottom of the tube. Remove the TE buffer from the bead pellet and repeat the wash a second time.

   NOTE: If your samples are not already in a low-retention tubes, transfer your samples during the TE wash step.
2. Remove as much liquid as possible without disturbing the bead pellet. A small amount of liquid remaining in the tubes is acceptable.
3. Add 100 µl of the ChIP Elute Resin slurry to each sample.

   NOTE:
   - Make sure to mix the ChIP Elute Resin slurry very well before pipetting (mix by inversion several times or vortex). The resin sediments very quickly and needs to be mixed between each pipetting. After initial resuspension, a vigorous swirl of the bottle should be sufficient.
   - To avoid clogging the pipette tip with resin, you may cut a few mm off the end of the tip.
   - 1–10% of the total chromatin input should be processed alongside the samples. Add 100 µl of the ChIP Elute Resin slurry to total chromatin input (up to 30 µl in a 1.5 ml low-retention tube) and process along with your ChIP samples.
4. Mix by tapping the tubes or gently vortexing.
5. Briefly spin the tubes to collect the liquid at the bottom of the tube.
6. Incubate at 95ºC for 10 min in the pre-heated heat block.

   NOTE: In this step, DNA is eluted from the Protein A/G beads and cross-linking is reversed.
7. Remove samples from the heat block and allow them to cool down at room temperature for about 1 min.
8. Briefly spin the tubes to collect the liquid at the bottom of the tube.
9. Add 2 µl of Proteinase K and mix by tapping the tubes or gently vortexing.
10. Briefly spin the tubes to collect the liquid at the bottom of the tube.
11. Incubate at 55ºC for 15 min in the pre-heated heat block.

    NOTE: This protocol has been tested with cultured 293T cells. Some samples may require a longer Proteinase K incubation. If needed, the incubation time can be increased to 1 hr or longer.
12. Move the samples to the 95ºC heat block and incubate for 10 min to inactivate Proteinase K.
13. Remove samples from the heat block and allow them to cool down at room temperature for about 1 min.
14. Briefly spin the tubes to collect the liquid at the bottom of the tube.

15. Remove approximately 100 µl of supernatant without disturbing the slurry pellet, and transfer to a new 1.5 ml tube. You may not be able to recover a full 100 µl of liquid; it is better to remove a slightly smaller volume than disturb the pellet.
16. Add 100 µl of DNA Dilution Buffer to the resin pellet and vortex for a few seconds
    NOTE: This step helps extract additional DNA remaining in the resin bed volume and improves yield.
17. Briefly spin the tubes to collect the liquid at the bottom of the tube.
18. Remove approximately 100 µl of supernatant and pool with the supernatant from Step 15.

NOTE:

- At this point samples can be stored at –20ºC for several weeks.
- If desired, enrichment of known targets can be verified by qPCR at this stage prior to further DNA purification.

IV. Protocol: DNA purification and concentration

1. To the approximately 200 µl of pooled supernatant, add 2 volumes (400 µl) of ssDNA Binding Buffer and mix by inverting or vortexing.
2. Place ssDNA Binding Columns into Collection Tubes and load the 600-µl samples from the previous step.
3. Centrifuge at 11,000 x g for 30 sec. Discard flowthrough and place the columns back into the collection tubes.
4. Add 600 µl of ssDNA Wash Buffer to the ssDNA Binding Columns.
   NOTE: Before the first use, add 100 ml of 100% ethanol to the ssDNA Wash Buffer.
5. Centrifuge at 11,000 x g for 30 sec. Discard flowthrough and place the columns back into the collection tubes.
6. Repeat the wash once.
7. Centrifuge at 11,000 x g for 2 min to completely dry the membrane in ssDNA Binding Columns.
8. Place the columns into 1.5 ml **low-retention** tubes.
9. Elute ssDNA by adding 23 µl of DNA Dilution Buffer. Incubate at room temperature for at least 2 minutes.

NOTE:

- Eluting with pre-heated (55°C or higher) DNA Dilution Buffer and incubating the columns for 5 min will improve recovery.
- Eluting with 23 µl of DNA Dilution Buffer will provide the right input volume (20 µl) for the DNA SMART ChIP-Seq Kit. If target enrichment verification is to be performed at this step, prior to library construction (recommended), then eluting twice with 15–20 µl per elution will maximize recovery and provide extra volume for qPCR. However, the DNA will be more dilute, which may lead to unreliable quantification.

10. Centrifuge at 11,000 x g for 1 min.
11. If desired, quantify the DNA using the Qubit 2.0 Fluorometer (Life Technologies) along with the Qubit ssDNA Assay Kit (Life Technologies, Cat. No. Q10212). If the input DNA concentration is too low to be quantified, use 17 or more PCR cycles for library amplification when using the DNA SMART ChIP-Seq Kit.
12. Store purified DNA at –20ºC. Although it should be stable for several months, ssDNA is more prone to degradation than dsDNA. For long term storage, store the samples at –80ºC.

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Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
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