

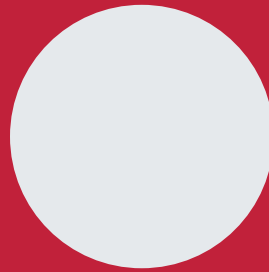
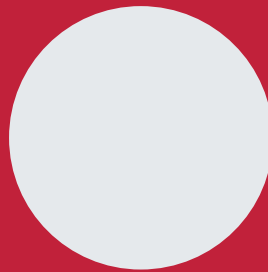
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Immunoprecipitation Buffers

(iDeal ChIP-seq Kit for TFs)

Cat. No. C01010174



Kit Materials

The content of this kit is sufficient to perform 24 chromatin immunoprecipitations (including IP, washes and elution). Please note that this kit does not contain magnetic beads, which can be ordered separately (protein A-coated: Cat. No. C03010020 / protein G-coated: C03010021). Store the components at the indicated temperature upon receipt.

Reagent	Quantity	Storage
5% BSA (DNA free)	780 μ l	-20°C
ChIP-seq grade water	27 ml	4°C
5x ChIP Buffer iC1b	6.8 ml	4°C
Wash buffer iW1	8.4 ml	4°C
Wash buffer iW2	8.4 ml	4°C
Wash buffer iW3	8.4 ml	4°C
Wash buffer iW4	8.4 ml	4°C
Elution buffer iE1	3.6 ml	4°C
Elution buffer iE2	144 μ l	4°C

Required Materials Not Provided

Reagents

- Protease Inhibitor Mix, 100 μ l (Cat. No. C12010011)
- DiaMag protein A-coated magnetic beads (ChIP-seq grade) (Cat. No. C03010020)
- DiaMag protein G-coated magnetic beads (ChIP-seq grade) (Cat. No. C03010021)
- Gloves to wear at all steps
- RNase/DNase-free 1.5 ml tubes

Equipment

- DiaMag1.5 magnetic rack (Cat. No. B04000003)
- Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
- DiaMag Rotator (Rotating wheel) (Cat. No. B05000001)
- Vortex
- Thermomixer

Protocol



The immunoprecipitation step can also be performed using the semi-automated **ChIPettor™ System**. If doing so, please refer to the corresponding protocol delivered with this product. Alternatively, the protocol can also be downloaded from the website www.diagenode.com

Immunoprecipitation (IP, washes, elution)

1. Dilute the **5x ChIP buffer iC1** with **ChIP-seq grade water** to obtain 4 ml of **1x ChIP buffer iC1**. Add 80 µl of **5% BSA**.
2. Take the required amount of **magnetic beads** (30 µl/ChIP) and wash them 3 times with 1 ml **1x ChIP buffer iC1b + BSA**.
3. Resuspend the beads in the original volume of **1x ChIP buffer iC1b + BSA**.
4. Prepare the following ChIP reaction mix:
 - 6 µl of **5% BSA**
 - 1.8 µl of **200x protease inhibitor cocktail**
 - 20 µl of **5x ChIP buffer iC1b**
 - 42.2 µl **ChIP-seq grade water**
 - 30 µl **DiaMag Protein A (or Protein G) - coated magnetic beads**
5. Add the required amount of **antibody**.
6. Incubate for 2-4 hours (or overnight) at 4°C on a rotating wheel.
7. Add 250 µl **sheared chromatin**. Keep 2.5 µl chromatin aside to serve as input.
8. Incubate overnight at 4°C on a rotating wheel.
9. The next day, briefly spin the tubes, place them in the ice-cold magnetic rack and discard the supernatant.
10. Add 350 µl ice-cold **Wash buffer iW1** and incubate for 5 min at 4°C on a rotating wheel. Discard the wash buffer using the Diagenode magnetic rack.
11. Repeat the wash as described above once with **Wash buffer iW2, iW3 and iW4** using the same buffer volume, respectively.
12. After removing the last wash buffer, add 100 µl of **Elution buffer iE1** to the beads and incubate for 30 min on a rotating wheel at room temperature.
13. Briefly spin the tubes and place them into the Diagenode magnetic rack. Transfer the supernatant to a new tube and add 4 µl of **Elution buffer iE2**. Also add 97.5 µl **buffer iE1** and 4 µl of **buffer iE2** to the 2.5 µl input sample. Incubate for 4 hours or overnight in a thermomixer at 1,300 rpm and 65°C.

For purification of your ChIP'd DNA we recommend to use the IPure kit (Cat. No. C03010011)

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