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Innovating Epigenetic Solutions

Immunoprecipitation Buffers

(iDeal ChIP-seq Kit for Histones)

Cat. No. **C01010173**

Version 2 I 04.16

MANUAL

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)	144 µl	-20°C
ChIP-seq grade water	8.4 ml	4°C
5x ChIP Buffer iC1	3.6 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 µl	4°C

Required Materials Not Provided

Reagents

- Protease Inhibitor Mix, 100 µl (Cat. No. C12010011)
- Sodium butyrate, 1 ml (Cat. No. C12020010)
- 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 **1x ChIP buffer iC1**. Place on ice.
2. Take the required amount of **DiaMag Protein A-coated magnetic beads** (20 µl/IP) and wash four times with twice the volume of ice-cold **1x ChIP buffer iC1**.
3. Resuspend the beads after the last wash in the original volume of **1x ChIP buffer iC1**.
4. Set aside 1 µl (1%) of the sheared chromatin to use as input sample and keep at 4°C.
5. Prepare the following ChIP reaction mix (1 IP):
   - 6 µl of **5% BSA**
   - 1.5 µl of **200x protease inhibitor cocktail**
   - 56 µl of **5x ChIP buffer iC1**
   - 100 µl of **sheared chromatin**
   - 20 µl of **DiaMag Protein A (or Protein G) - coated magnetic beads**
   - x µl **ChIP-seq grade antibody**
   - add **ChIP-seq grade water** to a total volume of 300 µl
     If required, NaBu (HDAC inhibitior, 20mM final concentration) or other inhibitors can also be added.
6. Incubate overnight at 4°C on a rotating wheel.
7. The next day, briefly spin the tubes, place them in the ice-cold magnetic rack and discard the supernatant.
8. 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**.
9. Repeat the wash as described above once with **Wash buffer iW2, iW3 and iW4** using the same buffer volume, respectively.
10. 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.
11. 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 99 µl **buffer iE1** and 4 µl of **buffer iE2** to the 1 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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DIAGENODE HEADQUARTERS

DIAGENODE S.A.
BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

DIAGENODE INC.
USA | NORTH AMERICA
400 Morris Avenue, Suite #101
Denville, NJ 07834
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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