diagenode

A Hologic Company

ChIP cross-link Gold

ChIP grade reagent | Separately available

Cat. No. C01019027

Version 1 | 03.19

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Content

Introduction

The first step of a ChIP assay is the cross-linking in order to fix the cells. Cross-linking is typically achieved by using formaldehyde which forms reversible DNA-protein links. Formaldehyde rapidly permeates the cell membranes and enables a fast cross-linking of closely associated proteins in intact cells. However, formaldehyde is usually not effective to cross-link proteins that are not directly bound to the DNA. For example, chromatin interactions with inducible transcription factors or with cofactors that interact with DNA through protein-protein interactions are not well preserve with formaldehyde. So, for higher order and/or dynamic interactions, other cross-linkers should be considered for efficient protein-protein stabilization such as the Diagenode ChIP cross-link Gold. This reagent is to use in combination with formaldehyde. The protocol involves a sequential fixation. A first protein-protein fixation by the ChIP cross-link Gold followed by protein-DNA fixation by formaldehyde.

Format: supplied as a 250 x concentrated solution

Shipping conditions: shipped at -20°C

Storage conditions: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Protocol

The protocol described below is for the use of ChIP cross-link Gold in association with the iDeal ChIP-seq kit for Transcription Factors (Cat. No. C01010054, C01010055 and C01010170) or Chromatin shearing optimization kit – Low SDS (for Transcription Factors) (C01020013)

STEP 1. Cell collection and DNA-protein cross-linking

1. Remove the medium from the flask and wash the cells two times with PBS (20 ml for a 175 cm² culture flask).
2. Add PBS/MgCl2 (PBS with 1 mM MgCl2) to the cells (20 ml for a 175 cm² culture fask).
3. Add 80 µl of ChIP cross-link Gold to the flask. Immediatly swirl to get the ChIP Cross-link Gold into solution. It may form a white precipitate after the addition of the ChIP Cross-link Gold. However it will be dissolved by gently mixing
4. Incubate at RT for 30 min. For optimal fixation, this timing may vary between 30 min and 45 min
5. Remove the fixating solution and wash 2 times with PBS.
6. Add 20 ml of PBS to the cells
7. Dilute formaldehyde in Fixation buffer to a final concentration of 11%
8. Add 2 ml of diluted formaldehyde to the 20 ml of PBS. Incubate the cells for 15 minutes at room temperature with gentle shaking. The fixation time can depend on your target of interest (between 10 and 20 min).
9. Add 1/10 volume of Glycine to the cell culture medium to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking

   Note: The fixed cells can be stored at -80°C for up to 4 months. However, we strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP for ChIP-sequencing.
10. Continue with "STEP 2. Cell lysis and chromatin shearing" of the iDeal ChIP-seq kit for Transcription factors or Chromatin shearing optimization kit – Low SDS (for Transcription Factors)

STEP 2. Cell lysis and chromatin shearing

For adherent cells (~25 million cells):

11a. Remove the medium and wash the cells once with 20 ml of PBS. Keep everything at 4°C from now on.

12a. Add 5 ml of cold lysis buffer iL1b to the plate and collect the cells by scraping.

13a. Rinse the flask with an additional 20 ml of buffer iL1b.

14a. Incubate at 4°C for 20 minutes.

For suspension cells (~25 million cells):

11b. Collect the cells by centrifugation at 1,600 rpm and 4°C for 5 minutes.

12b. Wash the cells once with 20 ml of PBS. Keep everything at 4°C from now on.

13b. Resuspend the cells in 25 ml of cold lysis buffer iL1b.

14b. Incubate at 4°C for 20 minutes.

15. Centrifuge at 1600 rpm and 4°C for 5 minutes. Discard the supernatant and resuspend the cells in 15 ml lysis buffer iL2. Incubate at 4°C for 10 minutes with gentle mixing.

16. Centrifuge at 1,600 rpm and 4°C for 5 minutes.

17. Add 1/200 volume protease inhibitor cocktail (PIC) to shearing buffer iS1b.

18. Discard the supernatant and resuspend the cells in shearing buffer iS1b + PIC to a final concentration of 15 million cells/ml. Resuspend by pipetting up and down.

19. Shear chromatin by sonication using the Bioruptor®. An initial time course experiment is recommended.
    - For Bioruptor® Standard or Plus use High power setting for 10-30 cycles (30 seconds ON, 30 seconds OFF). Stop the system after each run of 10 cycles, vortex and spin down sample.
    - For Bioruptor® Pico, sonicate samples for 5-15cycles (30 seconds ON, 30 seconds OFF). Vortexing is not required between runs.
20. Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin.

STEP 3. Sheared chromatin analysis

21. Take 50 µl of sheared chromatin for shearing assessment.
22. Add 2 µl of diluted RNase cocktail and incubate 1h at 37°C (dilution of 1µl of cocktail RNase in 150 µl of ChIP-seq grade water).
23. Add 50 µl of elution buffer iE1 and 4 µl of elution buffer iE2, mix thoroughly.
24. Incubate samples at 65°C for 4h (or overnight).
25. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the sample at RT for 10 min on a rotating wheel.
26. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
27. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.
28. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
29. Precipitate the DNA by adding 10 µl DNA precipitant, 5 µl of co-precipitant, and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
30. Centrifuge for 25 min at 13,000rpm at 4°C. Add 500 µl of ice-cold 70% ethanol to the pellet.
31. Centrifuge for 10 min at 13,000 rpm at 4°C. Air-dry the pellet.
32. Re-suspended the pellet in 20 µl of TE buffer.
33. Run samples in a 1.5% agarose gel.

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