INSTRUCTIONS

Pierce Chromatin Prep Module

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26158

Number	Description
26158	**Pierce Chromatin Prep Module**

Kit Contents:

Membrane Extraction Buffer, 10mL; store at 4°C

Nuclear Extraction Buffer, 10mL; store at 4°C

MNase Digestion Buffer, 5mL; store at 4°C

MNase Stop Solution, 0.5mL; store at 4°C

Halt™ Protease and Phosphatase Inhibitor Cocktail EDTA-free (100X), 4 × 90µL microtubes; store at 4°C

Glycine Solution (10X), 15mL; store at 4°C

PBS (20X), 15mL; store at 4°C

Micrococcal Nuclease (ChIP Grade) (10U/µL), 25µL; store at -20°C

Proteinase K, (20mg/mL), 0.25mL; store at -20°C

DTT, Lyophilized, 1 vial; store at room temperature or 4°C

Storage: Upon receipt store contents of yellow pouch at -20°C. Store all other components at 4°C. The kit is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Pierce™ Chromatin Prep Module provides a simple, fast and reproducible method to isolate chromatin for use in assays that monitor transcription regulation through histone modification[1] (epigenetics) or transcription factor-DNA binding interactions, such as chromatin immunoprecipitation (ChIP). The module contains all reagents necessary to isolate crosslinked chromatin from mammalian cells. The specially titrated and tested micrococcal nuclease digests the DNA, eliminating variable results caused by the traditional method of sonication. The advantages of enzymatic digestion include digestion reproducibility, reaction control, and easy titration of the enzyme for specific cell types. The included lysis reagents extract and solubilize the crosslinked DNA:protein complexes, providing simple and convenient isolation of chromatin-bound DNA without a dounce homogenizer. Because there is less than 15% contamination from other cellular compartments, the protein of interest can be efficiently enriched in downstream applications.

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Additional Materials Required

- Mammalian cell culture reagents and equipment
- Chemical fume hood
- Cell scrapers
- Thermomixer or heat block
- 50 and 15mL conical tubes
- 16% Formaldehyde (Product No. 28906)
- DMSO (optional)
- Sodium chloride (5M)
- Nuclease-free water

Protocol for Chromatin Prep Module

Note: Read the entire protocol before beginning the assay.

A. Experimental Design

- When designing a ChIP experiment, consider the number of immunoprecipitations desired from the chromatin sample and the number of cell culture conditions (e.g., drug-treated vs. non-treated). For accurate comparison, immunoprecipitate the same target proteins and controls from each cell culture condition.
- Performing a ChIP using normal rabbit IgG is an effective negative control. An abundant and ubiquitous DNA-binding protein, such as histone H3, can be used as a positive control.
- If performing multiple ChIPs from a single cell culture condition, crosslinked chromatin may be prepared in bulk. Scale the reagent amounts according to the number of ChIPs desired (Table 1) and sub-divide the chromatin sample after diluting with the IP Wash Buffer. The total input control obtained from the cell culture condition is common to this set of ChIPs.

Table 1. Reagent volumes to use based on the number of ChIPs.

Example number of HeLa cells	2×10^6	6×10^6	2×10^7	6×10^7
Number of ChIPs	1	3	10	30
1X PBS	2.1mL	6.3mL	21mL	63mL
Lysis Buffer 1	0.1mL	0.3mL	1mL	3mL
Lysis Buffer 2	0.05mL	0.15mL	0.5mL	1.5mL
MNase Digestion Buffer Working Solution	0.1mL	0.3mL	1mL	3mL
1X IP Dilution Buffer	0.45mL	1.2mL	4.5mL	12mL

B. Material Preparation

Note: The amounts listed below are for one ChIP. If you are preparing chromatin in bulk or performing multiple ChIPs, multiply the reagent amounts by the number of ChIPs being performed.

Glycine Solution (10X)	If precipitate formed, warm solution to 37°C in a water bath for 30 minutes and vortex before use.
PBS	If precipitate formed, warm solution to 37°C in a water bath and vortex before use. Dilute PBS (20X) to 1X with nuclease-free water.
1M DTT	Add 50µL of ultrapure water to the DTT. Store this solution at -20°C for up to 6 months.
Lysis Buffer 1	Add 1µL of the Halt Cocktail to 100µL of Membrane Extraction Buffer in a microcentrifuge tube and place on ice.
Lysis Buffer 2	Add 0.5µL of the Halt Cocktail to 50µL of Nuclear Extraction Buffer in a microcentrifuge tube and place on ice.

MNase Digestion Buffer Working Solution	Add 0.1µL of 1M DTT to 100µL of 1X MNase Digestion Buffer and place tube at room temperature.

C. Crosslinking and Cell Pellet Isolation

Note 1: Perform all centrifugations at room temperature.

Note 2: This procedure is for one ChIP. Multiply reagent amounts by the number of ChIPs being performed (see Table 1).

1. Culture adherent mammalian cells and treat as desired.

   Optional: If you are unfamiliar with cell type being used, culture an extra dish of cells for determining cell number. Before crosslinking, trypsinize and determine the cell number of the extra dish of cells.

2. To each dish containing cell culture media, add sufficient quantity of 16% formaldehyde to obtain a final concentration of 1% formaldehyde.

   Caution: Formaldehyde is a skin irritant and the fumes are toxic. Use proper personal protective, laboratory safety and disposal equipment.

3. Mix well by gently swirling the dish. Incubate at room temperature for 10 minutes in a chemical fume hood.
4. To each dish containing cell culture media and formaldehyde, add Glycine Solution (10X) to a final concentration of 1X. Mix well by gently swirling the dish. Incubate at room temperature for 5 minutes in the chemical fume hood.
5. Aspirate formaldehyde/glycine-containing media in the fume hood. Properly dispose the formaldehyde-containing waste.
6. Wash the cells twice with one media volume of ice-cold PBS, removing each wash by aspiration.
7. Add 10µL of the Halt Cocktail to 1mL of ice-cold PBS. Add the solution to the cells, and detach cells by scraping. Transfer the cell suspension to a 1.5mL microcentrifuge tube using a pipette.
8. Centrifuge tubes at 3000 × *g* for 5 minutes.
9. Remove the PBS. Store the cell pellet(s) at -80°C, or proceed directly to Section C: Lysis and MNase Digestion.

D. Lysis and MNase Digestion

Note: For best results, empirically determine the optimal crosslinking time and Micrococcal Nuclease digestion conditions for each cell type (see Appendix A).

1. Use the crosslinked HeLa cells prepared above. Thaw stored cells on ice.
2. Add100µL Lysis Buffer 1 containing protease inhibitors to the cell pellet and pipette up and down to break up the pellet. Vortex the tube for 15 seconds and incubate on ice for 10 minutes.
3. Centrifuge at 9000 × *g* for 3 minutes and remove the supernatant.
4. Resuspend nuclei in 100µL of MNase Digestion Buffer Working Solution.
5. Add 0.25µL of Micrococcal Nuclease (ChIP Grade) (10U/µL), vortex the tube and incubate in a 37°C water bath for 15 minutes, mixing by inversion every 5 minutes.
6. Add 10µL MNase Stop Solution to stop the reaction, vortex briefly and incubate on ice for 5 minutes.
7. Centrifuge at 9000 × *g* for 5 minutes to recover the nuclei. Remove the supernatant.
8. Resuspend nuclei in 50µL of Lysis Buffer 2 containing protease/phosphatase inhibitors and incubate on ice for 15 minutes, vortexing for 15 seconds every 5 minutes.
9. Centrifuge at 9000 × *g* for 5 minutes and transfer the supernatant, containing the digested chromatin, to a new 1.5mL tube. Proceed to the immunoprecipitation or store samples at -80°C.

Troubleshooting

Problem	Possible Cause	Solution
Chromatin fragments are too large (> 1000 base pairs)	Crosslinking time is too long	Shorten crosslinking time
	Cell to Micrococcal Nuclease (MNase) ratio is too high	Increase amount of MNase or decrease cell number (refer to the MNase digestion optimization protocol in Appendix A)
Chromatin fragments are too small (< 200 base pairs)	Cell to MNase ratio is too low	Decrease amount of MNase or increase cell number (refer to the MNase digestion optimization protocol in Appendix A)

Appendix A: Optimization of micrococcal nuclease digestion

1. Prepare crosslinked cell pellet from 10^7 cells as described in Section C.
2. Follow the lysis procedure in Section D steps 1-4; however, use 0.5mL of the Lysis Buffer 1, Membrane Extraction Buffer and MNase Digestion Buffer Working Solution.
3. Transfer 100µL of the resuspended nuclei into five tubes (2×10^6 nuclei/tube), labeling the tubes 0, 1, 2, 4 and 6.
4. Add 2µL of the Micrococcal Nuclease (ChIP Grade) to 18µL of MNase Digestion Buffer (1:10 dilution).
5. Add 0, 1, 2, 4 and 6µL of the diluted Micrococcal Nuclease to the corresponding tubes of nuclei and incubate for 15 minutes in a 37°C water bath mixing by inversion every 5 minutes.
6. Add 20µL of MNase Stop Solution to each tube and incubate on ice for 5 minutes.
7. Centrifuge at 9000 × *g* for 5 minutes to recover the nuclei and remove the supernatant.
8. Resuspend nuclei in 50µL of Lysis Buffer 2 and incubate on ice for 15 minutes, vortexing for 15 seconds every 5 minutes.
9. Centrifuge at 9000 × *g* for 5 minutes.
10. Prepare five 1.5mL microcentrifuge tubes labeled 0, 2, 4 and 6 each containing 6.6µL Nuclease Free Water, 2.4µL of 5M NaCl, and 1µL Proteinase K (20mg/mL).
11. Transfer 50µL of each nuclear supernatant, containing digested chromatin, to the corresponding tube.
12. Vortex each tube for 10 seconds and incubate the tubes at 65°C in a heat block for 1.5 hours.
13. Analyze 15-25µL of each sample and DNA size marker by agarose gel electrophoresis. Optimal digestion should yield fragments from 200 to 1000 base pairs with a more intense ladder of bands occurring at approximately 160, 320 and 480 base pairs, which corresponds to the 1, 2, and 3 nucleosome units.

Related Thermo Scientific Products

See our website for a listing of ChIP-validated antibodies.

28908 **16% Formaldehyde (w/v), Methanol-free,** 10 × 1mL
20688 **Dimethylsulfoxide (DMSO), Sequanal grade,** 950mL
26158 **Pierce Chromatin Prep Module**
26159 **ChIP Grade Protein A/G Plus,** 0.65mL
26160 **Proteinase K Solution (20mg/mL),** 0.25mL
28372 **BupH™ Phosphate Buffered Saline Pack,** 40 packs
78425 **Halt Protease Inhibitor Cocktail, EDTA-Free (100X),** 24 vials
78443 **Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (100X),** 24 vials
78833 **NE-PER Nuclear and Cytoplasmic Extraction Kit**
20148 **LightShift™ Chemiluminescent EMSA Kit**
23225 **Pierce BCA Protein Assay Kit**
22662 **Pierce 660nm Protein Assay Kit**

AB1219	**Absolute™ QPCR SYBR™ Green Fluorescein Mix**
AB-0900/w	**Thermo-Fast™ 96 Semi-Skirted PCR Plate, opaque white**
AB-1170	**ABsolute QPCR Seal**

Cited References

1. Lee, D.Y., *et al.* (2009). Role of protein methylation in regulation of transcription. *Endocrine Rev* **26(2):**147-70.

General References

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