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Double-fixation prior to ChIPs

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol is for the double-fixation of chromatin regulators for chromatin precipitation. The double-fixation method can improve signal-to-noise ratios of chromatin regulator ChIPs, especially for transiently bound proteins or those found in large protein complexes.

Materials

Disuccinimidyl glutarate (DSG) [ChemCruz]

DMSO [Sigma]

PBS [Fisher Scientific]

2.5M Glycine Solution: Dissolve 93.84g Glycine (MW: 75.07g/mol) in 400ml sterile water. Adjust final volume to 500 ml and sterile filter

CiA Fix Buffer: 25 ml 1M HEPES pH 8.0; 1 ml 0.5M EDTA; 0.5 ml 0.5M EGTA; 10 ml 5M NaCl Adjust final volume to 500 ml with sterile water and filter sterilize

trypsin-EDTA 0.25% (Fisher Scientific)

16% Formaldehyde Solution (w/v) Methanol-Free [Thermo Scientific]

Safety warnings

Use proper safety precautions when using formaldehyde.

Fresh DSG

30m

1 Place DSG at room temperature for approximately 30 minutes before weighing it out. 30m

2 Measure DSG and dissolve in DMSO accordingly (using approximately 80 ul of solution per sample) 5m

Number of Samples	DSG (mg)	Vol. DMSO (ul)
2	13	160
4	25	320
6	38	480
12	72	960

Isolate and count single cells

20m

3 Harvest the cells by first washing with PBS 1m

4 Incubate cells in trypsin-EDTA 0.25% until dissociated 5m

5 Quench trypsin with media + FBS and move cells to a 15ml conical. Spin down at 300g for 4 minutes 4m

6 Resuspend cells in PBS and count 5m

7 Move 30e6 cells to a new tube and bring the volume up to 10mls with PBS and spin down at 300g for 4 minutes — 4m

8 Resuspend cells in 10mls fresh PBS — 1m

DSG fixation (First) — 36m

9 Add 80 ul DSG stock to each sample in 10 ml of PBS and incubate for 30 minutes at room temperature with rocking.
Note: After incubation with DSG, cells may adhere to the walls of pipette tips, so following
this incubation, it is recommended that tips be coated with 1% BSA/PBS — 30m

10 Pellet samples by spinning at 1500 x g for 10 minutes at 4C — 4m

11 Carefully aspirate the supernatant, taking care not to disturb the pellet. — 1m

12 Resuspend each sample in 10 ml of CiA Fix Buffer — 1m

Formaldehyde Fixation (Second) — 34m

13 Add 667 ul of 16% Formaldehyde to the sample dropwise and incubate for exactly 10 minutes at room temperature using rotation — 10m

14 Stop cross-linking by adding 555 ul of 2.5M glycine. — 1m

15 Incubate samples on ice for 5 minutes — 5m

16 Pellet samples by spinning at 1500 x g for 10 minutes at 4°C — 10m

17 Aspirate supernatant taking care not to disturb the pellet — 1m

18 Wash fixed cells with 10ml PBS 1m

19 Pellet samples by spinning at 1000 x g for 5 minutes at 4C and aspirate supernatant. 5m

20 Samples can be stored at -80°C (snap freeze) or can begin processing for chromatin immunoprecipitation 1m

Protocol references

Adapted from B.Tian et al., 2012, pp. 106-108
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