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

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Double-fixation prior to ChIPs

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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.

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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. 30m

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

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