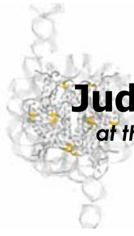

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Chromatin Fibers from Mammalian Tissue Culture Cells

Revised by J Sims from Susan Forsburg
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Procedure

1. Trypsinize cells, wash in PBS and resuspend in NIB buffer. Spin down the cells 600xg 7 minutes at 4 deg. Wash nuclear pellet twice with PBS+1mM PMSF.
2. Resuspend nuclear pellet in chromatin lysis buffer and pipet lines of chromatin down supercharged slides. Let dry for ~5 minutes.
   I usually draw two lines down the width of the slide using a liquid blocker marker (PAP PEN) before streaking the chromatin onto the slides. This prevents the antibody from moving beyond where the chromatin fibers should be
3. Immerse slides in chromatin lysis buffer in a Coplin jar for 10 minutes.
4. Slowly lift the slides straight out of the jar and immerse in PBS for ~2 minutes
5. Immerse slides in 4% formaldehyde/PBS for 20 minutes
6. Slowly lift the slides straight out of the jar and immerse in PBS for ~10 minutes
7. Immerse slides in blocking solution for 1 hour at room temperature
8. Slowly lift the slides straight out of the jar and blot the ends of the slides to remove excess moisture. Add 100uL primary antibody, diluted in blocking solution, to the slides and incubate overnight at 4 deg.
9. Immerse slides in wash buffer 3x 5 minutes at room temperature
10. Add 100uL secondary antibody, diluted in blocking solution, to the slides and incubate at room temperature for 1 hour. Make sure to cover the slides with foil to protect from the light.
11. Immerse slides in wash buffer 3x 5 minutes at room temperature
12. Add mounting medium to the slides and a coverslip.

Solutions

NIB Buffer

150mM NaCl
10mM HEPES, pH 7.4
1.5mM MgCl2
10mM KCl
0.5% NP-40
0.5mM DTT
1mM PMSF
1ug/mL pepstatin
1ug/mL aprotinin/leupeptin

Chromatin Lysis Buffer

25mM Tris, pH 7.5
0.5M NaCl
1% Triton X-100
0.5M Urea (diluted from 1M urea made up immediately prior to use)

Blocking Solution (in PBS)

1% BSA
0.5% Triton X-100
0.02% NaN3

Wash Solution

0.05% Tween 20 in PBS

**For detecting more loosely associated proteins, use Protein Lysis Buffer (200mM Tris, pH 7.5, 50mM EDTA, and 0.5% SDS) instead of chromatin lysis buffer.