

ChIP from plant material with the LowCell# ChIP Kit

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As preparation of plant material for Chromatin Immunoprecipitation differs essentially from preparation of culture cells, the LowCell# ChIP protocol has to be adapted respectively. We recommend therefore to follow the first steps of a ChIP protocol optimized for plant material¹ and then to go ahead with the LowCell# ChIP. Of note, reagents for the chromatin crosslinking and preparation are not included in the LowCell# ChIP Kit.

1. Chromatin Crosslinking

The LowCell# ChIP Kit permits you to use as few as 0.1g plant material per ChIP experiment, instead of 1.5g as mentioned in this Plant ChIP protocol. We propose to use 0.1g of plant material per experiment which can be used for 9 IPs + 1 input or 4 IPs + 1 input, whereby the rest can either be used for sheared chromatin analysis. Volumes are scaled accordingly.

1. Harvest 0.1g seedlings (3-4 week old seedlings) and place them into a 50 ml Falcon tube;
2. Rinse seedlings twice with 40ml bidistilled water. Remove as much water as possible after second wash;
3. Add 10ml 1% formaldehyde solution. Gently submerge seedlings at the bottom of the tube by stuffing the tube with nylon mesh. Screw on cap and poke cap with needle holes. Put in exsiccator and draw vacuum for 10 minutes;
4. Release vacuum slowly and shake exsiccator slightly to remove air bubbles. Seedlings should appear translucent;
5. Add 625 µl 2M glycine to quench crosslinking (final concentration 0.125M). Draw vacuum for 5 minutes;
6. Again, release vacuum slowly and shake exsiccator slightly to remove air bubbles;
7. Remove nylon mesh, decant supernatant and wash seedlings twice with 40ml of bidistilled water; After second wash, remove as much water as possible and put seedlings between two layers of kitchen paper. Roll up paper layers carefully to remove as much liquid as possible.

At this step, plant material can be shock-frozen in liquid nitrogen and stored at -80°C. In my hands, this step provides the only possibility, where the protocol can be interrupted and still gives reproducible results.

2. Chromatin preparation

1. Precool mortar with liquid nitrogen. Add 2 small spoons of silicon dioxide (Sigma, S9887) and plant material. Grind plant material to a fine powder;
2. Use cooled spoon to add powder to 10ml of Extraction Buffer 1 stored on ice. Vortex to mix and keep at 4°C until solution is homogenous;
3. Filter extract through Miracloth into a new, ice-cold 50ml Falcon tube. Rigidly press out solid material;
4. Repeat step 3;
5. Centrifuge extract using the Beckman JS 7.5 rotor (or equivalent) at 4000 rpm for 20 minutes at 4°C;
6. Gently pour off supernatant and resuspend pellet in 1ml of Extraction Buffer 2 by pipetting up and down. Transfer solution to Eppendorf tube;
7. Spin in cooled benchtop centrifuge at 13000 rpm for 10 minutes;
8. Remove supernatant and resuspend pellet in 300 µl of Extraction Buffer 2 by pipetting up and down;
9. Add 300µl of Extraction Buffer 3 to fresh Eppendorf tube; Use pipette to carefully layer solution from step 8 onto it. Spin in cooled benchtop centrifuge at 13000 rpm for 1 hour. Place the Buffer B (LowCell# ChIP Kit) at room temperature (RT) before use. Make sure when working with this Buffer, that there are no crystals left in solution. Otherwise heat up gently and mix until complete disappearance of such crystals.
10. Remove supernatant.

Start now with LowCell# ChIP Kit at "STEP 3. Cell Lysis, chromatin shearing", point 19. Sonicate chromatin from 0.1g plant material in 130 µl of Buffer B.

¹Chromatin Immunoprecipitation Protocol to Analyze Histone Modifications in *Arabidopsis thaliana* (PROT12), http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=3246, Werner Aufsatz, 2007.

Additional Reagents (not provided with LowCell# ChIP Kit)

Extraction Buffer 1

0.4M Sucrose
10mM Tris-HCl, pH 8.0
10mM MgCl₂
5mM β-mercaptoethanol, with Protease Inhibitors

Extraction Buffer 2

0.25M Sucrose
10mM Tris-HCl, pH 8.0
10mM MgCl₂
1% Triton X-100
5mM β-mercaptoethanol, with Protease Inhibitors

Extraction Buffer 3

1.7M Sucrose
10mM Tris-HCl, pH 8.0
2mM MgCl₂
0.15% Triton X-100
5mM β-mercaptoethanol, with Protease Inhibitors