Lysis

Tuesday, April 18, 2017 3:00 PM

Lysis is essentially composed of three steps:

1. Add lysis buffer
2. Disrupt tissue/cells
3. Clear cell debris

The lysis buffer is optimized according to sample type and experiment type. Some common situations are outlined below.

Insect Cells

Native Lysis buffer: 50 mM Tris-HCl or NaH2P)4, 500 mM NaCl, 0.5% NP-40 (pH ~7.4-7.5)
Optional additives: Phosphatase inhibitor cocktails 2 and 3, imidazole (less than 10 mM)

Denaturing Lysis buffer: 7 M urea, 100 mM NaH2PO4, 10 mM Tris-HCl, pH 8.0

1. Remove frozen cells from -80 C right before lysis
2. Add cold lysis buffer to cell pellet and resuspend by pipetting up and down gently
3. Leave on ice for 5-10 min
4. Sonicate samples 3x for 5 s each (allow a 15-20 s "cool-down" period between each burst)--leave samples on ice at all times, especially for phosphorylation work
5. Spin down samples at 16000 x g for 10 min at 4 C
6. Transfer cleared supernatant to a new eppendorf, being careful not to disturb the cell pellet at the bottom of the tube

Mammalian cells

Lysis buffer: 100 mM Tris-HCl, 150 mM NaCl, 1% NP-40 (pH ~7.4-7.5)
Optional additives: Phosphatase inhibitor cocktails 2 and 3, 10 mM NaF, 1 mM Na3VO4, protease inhibitor cocktail

1. Remove frozen cells from -80 C right before lysis
2. Add cold lysis buffer to cell pellet and resuspend by pipetting up and down gently
3. Leave on ice for 5 min
4. Sonicate samples 3x for 5 s each (allow a 15-20 s "cool-down" period between each burst)--leave samples on ice at all times, especially for phosphorylation work
5. Spin down samples at 16000 x g for 10 min at 4 C
6. Transfer cleared supernatant to a new eppendorf, being careful not to disturb the cell pellet at the bottom of the tube

Mammalian tissue for digestion

Lysis buffer: 100 mM Tris-HCl (pH 8.5), 6 M guanidine HCl, 40 mM 2-chloroacetamide, 10 mM TCEP
Optional additives: Phosphatase inhibitor cocktails 2 and 3, 10 mM NaF, 1 mM Na3VO4, protease inhibitor cocktail

1. Prepare fresh lysis buffer and put on ice
2. Put dounce homogenizers on ice
3. Add a small amount of lysis buffer to each homogenizer and then transfer the tissue over. Add the rest of your lysis buffer on top of the tissue

4. Grind the tissue with 10-20 strokes of the homogenizer. If the tissue is difficult to break up, use more lysis buffer and/or additional grinding. Be careful not to introduce too many bubbles during homogenization.
5. Transfer the liquid to clean eppendorfs
6. Heat samples to 95 C for 5 min to denature proteins/reduce and alkylate cysteine residues
7. Cool on ice for 2 min
8. Sonicate samples 3x for 5 s each (allow a 15-20 s "cool-down" period between each burst)
9. Heat samples to 95 C for 5 min
10. Clear lysate by centrifuging for 20 min at 16000 x g at 4 C
11. Transfer cleared supernatant to a new eppendorf

If the tissue is to be used for something other than digestion/mass spectrometry (e.g. IP or western blot):

1. Remove GuHCl, CAA, and TCEP from lysis buffer. Substitute with RIPA buffer or mammalian lysis buffer containing a different detergent like Triton X-100
2. Skip the heating steps

E. coli

Lysis buffer: 50 mM Na phosphate, 300 mM NaCl, 1 mg/mL lysozyme (pH 7.5-8.0)
Optional additives: protease inhibitors, 5 mM imidazole (for His purification), 1 mM EDTA (not for His purification), nuclease such as DNase1

1. Add lysozyme to lysis buffer just before use and leave on ice
2. Add lysis buffer to E. coli pellet and pipette up and down to resuspend it
3. Leave samples on ice for 10 min
4. Sonicate samples 3x for 5 s each (allow a 15-20 s "cool-down" period between each burst)--leave samples on ice at all times
5. Spin down samples at 16000 x g for 10 min at 4 C
6. Transfer cleared supernatant to a new eppendorf, being careful not to disturb the cell pellet at the bottom of the tube

Plant tissue

Lysis buffer: 6M guanidine-HCl, 10 mM TECP, and 40 mM CAA in 100 mM Tris-HCl (pH 8.5)
Optional additives:protease inhibitor cocktail, 10 mM NaF, and phosphatase inhibitor cocktail 2 or 3

1. Prepare fresh lysis buffer and put ion ice
2. Weight the plant tissue.
3. Add a scoop of liquid nitrogen to mortar and transfer the plant tissue into the mortar.
4. Grind the tissue with pestle around 10 times, and add a scoop of liquid nitrogen. Repeat this step twice.
5. Add the amount of lysis buffer to the mortar and to reach the final concentration is 1 g/mL.
6. Wait few minutes until the frozen tissue powders melt, and grind the powders 10 times
7. Transfer the green liquid to clean eooendorfs
8. Heat samples at 95 C degrees for 5 min
9. Cool on ice for 5 min
10. Sonicate samples 3X for 10 s each(the amplitude is 30, and allow a 10 s cool down between each pulse)
11. Heat samples at 95 C degree for 5 min
12. Centrifuge the samples for 15 min at 16,000 x g at 4 C degree.
13. Transfer the supernatant to a new eppendorf for further methanol-chloroform precipitation