CSF Extract Prep for Spindle Assembly

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This protocol is essentially as described by Murray (1991), Cell Cycle Extracts. In Methods in Cell Biology, B.K. Kay and B. Peng, eds. (San Diego: Academic Press), pp. 581-605. I've included a protocol which emphasizes the points that we find are most important for obtaining good CSF extracts that are competent for CSF spindle assembly and for cycled spindle assembly. The indicated buffer amounts are sufficient for a 4 frog prep. For sperm nucleus preparation, see above protocol. For spindle assembly, see Sawin and Mitchison (1991) J. Cell Biol. 112: 925-940.

Notes Before Beginning: The quality of the eggs is essential for good CSF extracts. Always sacrifice quantity for quality when trying to make functional extracts. Discard any batches of eggs that have 'puff balls' or activated eggs as more than 10% of the eggs. We routinely use laid eggs and collect at about 16-17 hours. If you are trying to make extracts that will form spindles competent of anaphase chromosome segregation, we find it necessary to use only freshly squeezed eggs. Keep the eggs cool (16 deg C incubator) and only bring them to RT right before you are ready to prepare the extract.

Things You Need

Stock Buffers:

20X XB Salts:
2 M KCl
20 mM MgCl2
2 mM CaCl2
store at 4 deg C.

2 M Sucrose:
Sterile filter and store in aliquots at - 20 deg C.

1 M HEPES, pH 7.7:
Sterile filter and store at 4 deg C.

0.5 M K-EGTA, pH 7.7:
Sterile filter and store at RT.

Extract Prep Buffers:

MMR:
5 mM HEPES, pH 7.8
0.1 mM EDTA
100 mM NaCl
2 mM KCl
1 mM MgCl2
2 mM CaCl2
make 2 l- store at RT.

XB:
10 mM HEPES, pH 7.7
1 mM MgCl2
0.1 mM CaCl2
100 mM KCl

50 mM sucrose
make 250 ml- make fresh.

CSF-XB:
10 mM HEPES, pH 7.7
2 mM MgCl2
0.1 mM CaCl2
100 mM KCl
5 mM EGTA
50 mM sucrose
make 250 ml- make fresh.

Dejellying solution:
2 % cysteine; 1X XB salts, pH 7.8
water to 200 ml- make within 1 hour of use.

Energy Mix:
150 mM creatine phosphate
20 mM ATP
2 mM EGTA
20 mM MgCl2
100 ul aliquots- store at -20 deg C.

Equipment

- 1 600 ml beaker
- 1 150 X 75 mm petri dishes
- 5% gelatin in ddH20 (at 37 !C)
- Flame polished cut-off pasteur pipettes (diameter of opening approx. 2-3 mm)
- LPC (10 mg/ml each of leupeptin, pepstatin, chymostatin in DMSO)
- Cytochalasin D (10 mg/ml in DMSO)
- 13 X 51 mm ultraclear tubes
- SW55.1 @ 16 deg C in ultra

Procedure

Before Starting

1. Get all solutions ready and tubes in the rack
2. Have gelatin @ 37 deg C
3. Coat petri dish with 100 ul/dish of gelatin, swirl and replace with XB
4. Bring frogs to room temp at the last minute

Protocol

1. Collect laid eggs: keep eggs in separate batches if distinguishable difference in quality
2. Wash eggs in MMR till all the crap and dirt is removed in 600 ml beaker.
3. Garden away the bad eggs (pick out individually with pasteur)
4. Remove as much MMR as possible.
5. Dejelly in 2% cysteine till packed (~ 5 min)- remove all cysteine
6. Wash dejellied eggs 2-3 X with XB in gelatin-coated petri dish-remove all XB. For each wash swirl the eggs around the dish and then let the eggs settle back down. They should pack tightly after the jelly coat is removed.
7. Wash 2-3 X in CSF-XB (150 ml total volume)- remove as much buffer as possible.
8. Wash 2X in CSF-XB + 10 5g/ml PIs (100 ml total volume)
9. Transfer into 1 ml of CSF-XB + PIs + 100 ug/ml cytochalasin D in 13 X 51 ultraclear tubes (let eggs drop in)
10. Suck off all buffer from top (pretty dry)
11. Put into falcon tube and spin for 10 sec @ #4 in a clinical centrifuge.
12. Remove all buffer (pretty dry) and put in 1 ml versilube
13. Spin at #5 for 30 sec and full speed for 15 sec in a clinical centrifuge.
14. Remove all buffer and versilube (as dry as possible)

15. Crush @ 16 deg C: 15 min @ 10,000 rpm (full brake) in an SW55 rotor. We find that using the ultracentrifuge at this step gives much more reproducible extracts.
16. Collect extract with 18 gauge needle by puncturing the side of the tube and gently sucking out the cloudy cytoplasmic layer. You should be able to obtain about 0.5-0.75 ml of extract/tube.
17. Add 1/1000 volume of LPC and cyto D; 1/20 vol of 20X energy mix; 1/40 vol 2M sucrose.

Extract is Ready to go!