CSF 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)

20X XB Salts: (2 M KCl; 20 mM MgCl2; 2 mM CaCl2)
   KCl (74.55 g/mol): 37.3 g
   MgCl2 (4.9 M): 1.02 ml
   CaCl2 (111 g/mol): 0.055 g
water to 250 ml, sterile filter and store at 4 °C.

2 M Sucrose:
   Sucrose (342.3 g/mol): 171.5 g
water to 250 ml- sterile filter and store in aliquots at - 20° C.

1 M HEPES, pH 7.7:
   HEPES (238.3 g/mol): 35.7 g
water to 150 ml- sterile filter and store at 4 °C.

0.5 M K-EGTA:
   EGTA (380.4 g/mol): 19.02 g
pH to 7.7 with KOH- water to 100 ml, sterile filter and store at RT.

XB: (10 mM HEPES, pH 7.7; 1 mM MgCl2; 0.1 mM CaCl2; 100 mM KCl; 50 mM sucrose)
   20X XB Salts: 25 ml
   2 M Sucrose: 12.5 ml
   1M HEPES, pH 7.7  5 ml
   10 N KOH  55 µl
water to 500 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)
   XB: 100 ml
   MgCl2 (1 M):100 µl
   K-EGTA (0.5 M):  1 ml

Dejellying solution: (2 % cysteine; 1X XB salts)
   Cysteine: 4 g
   20X XB salts: 10 ml
   10 N NaOH: 0.9 ml
water to 200 ml.

NaBRB-80: (80 mM Pipes, pH 6.8; 1 mM MgCl2; 1 mM EGTA)
   Pipes (302.4 g/mol): 12.08 g
   MgCl2 (4.9 M):  102 µl
   EGTA (380.4 g/mol): 0.19 g
pH to 6.8 with NaOH, water to 500 ml- sterile filter and store at 4 °C.

Energy Mix: (150 mM creatine phosphate; 20 mM ATP; 2 mM EGTA; 20 mM MgCl2)
   Creatine phosphate (327.2 g/mol): 32.7 mg
   Na2-ATP (551.1 g/mol): 55.1 mg
EGTA (0.5 M): 20 µl
MgCl₂ (1 M): 10 µl
水 to 5 ml, aliquot in 100 µl aliquots and store at -20 °C.

Spindle Fix:
- 100% glycerol: 5.36 ml
- 25X MMR: 400 µl
- 10 mg/ml Hoechst: 1 µl
- water: 1.95 ml

Aliquot 192.5 µl/tube. Add 57.5 µl 16% formaldehyde before use.

**Equipment**
- 3-4 small petri dishes, rinsed in ddH₂O
- 3-4 medium petri dishes, rinsed in ddH₂O
- 5% gelatin in ddH₂O (at 37 °C)
- 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 A 16 °C in ultra

**Before Starting**
1) Get all solutions ready and tubes in the rack
2) Have gelatin @ 37 °C
3) In medium petri dishes put a small amount of XB till it covers the bottom
4) Have MMR in small petri dishes
5) Coat the petri dishes with 30 µl/ dish of gelatin, swirl and replace with XB)
6) Bring frogs to room temp at the last minute

**Protocol**
1) Squeeze frogs into small petri dishes
2) Collect laid eggs: keep eggs in separate batches if distinguishable difference in quality
3) Wash eggs in MMR till all the crap and dirt is removed
4) Garden away the bad eggs
5) Dejelly in 2% cysteine till packed (= 5 min)- remove all cysteine
6) Wash dejellied eggs 3-4X with XB in gelatin-coated petri dishes-remove all XB
7) Wash 2X in CSF-XB + PIs
8) Transfer into 1 ml of CSF-XB + PIs + 100 µg/ml cytochalasin D in 13 X 51 ultraclear tubes (let eggs drop in)
9) Suck off all buffer from top (pretty dry)
10) Put into falcon tube and spin for 10 sec @ #4
11) Remove all buffer (pretty dry) and put in 1 ml versilube
12) Spin at #5 for 30 sec and full speed for 15 sec
13) Remove all buffer and versilube (as dry as possible)
14) Crush @ 16 °C: 15' @ 10,000 rpm: Decel = 5 (very cloudy extract)
   15' @ 12,500 rpm: Decel = 5 (a cleaner extract)
15) Collect extract with 18 gauge needle
16) Add 1/1000 volume of LPC and cyto D; 1/20 vol of 20X energy mix; 1/40 vol 2M sucrose.

Rd tubulin (use clear tubes in H8)
Sperm nuclei in H1