Preparing CSF-extract from *Xenopus* Eggs

This protocol is essentially as described by Murray in „*Cell cycle extracts*“, Methods Cell Biol. 1991;36:581-605.

Frogs

HCG, SIGMA (in ddH_20, filter sterilize, keep at 4ºC)
PMSG, Intervet (optional)
Syringes
Needles ∅ 0.4 mm (e.g. Microlance™ sterile needles, Becton Dickinson)
Containers
Room / Incubator at 16ºC

- 1st injection: 50 U of human chorionic gonadotropin (HCG, SIGMA)
- alternatively: 50-100 U of pregnant mare serum gonadotropin (PMSG, Intervet)
- 2nd injection: 500 - 1000 U of HCG, 16 hours prior to when eggs are needed
- keep frogs seperated in containers in 1 x MMR at 16ºC

Solutions

Stock solutions

Cytochalasin B (10 mg/ml in DMSO; use 1:1000) SIGMA C2743
Cycloheximide (100 mg/ml in DMSO/EtOH; use 1:1000) Sigma C7698
Complete EDTA-free Protease Inhibitor (in ddH_20; use 1:100) Roche
(keep at -20ºC)

EDTA 0.5 M
EGTA 0.5 M
$MgCl_2$ 1M stock
$CaCl_2$ 1M stock
(filter sterilize, keep at RT)

10 x MMR	5 l
1 M NaCl	292.2 g
20 mM KCl	7.46 g
10 mM $MgCl_2$	10.17 g
20 mM $CaCl_2$	11 g
1 mM EDTA	10 ml (0.5 M stock)
50 mM HEPES pH 7.8	59.58 g
store at RT	

20 x XB-salts	1 l
2 M KCl	149.12 g
20 mM $MgCl_2$	20 ml (1M stock)

2 mM $CaCl_2$	2 ml (1M stock)

filter sterilize, keep at 4ºC

Prepare freshly

CSF-XB	1 l
10 mM HEPES	2.38 g
50 mM Sucrose	17.11 g
100 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$	50 ml (20 x XB-salts)
5 mM EGTA	10 ml (0.5 M stock)

adjust to pH 7.7 with KOH

Dejelly Buffer	1 l
2% (w/v) L-Cysteine	20 g
0.25 x MMR	25 ml (10 x MMR)

adjust to pH 7.8 with 5N NaOH

Extract preparation

Plastic Pasteur pipettes (e.g. Peske # 18-4321; 3 ml Pasteur-Pipetten graduiert)
Clinical centrifuge
Beckman Ultra-Clear centrifuge tubes (13 x 51 mm; Reorder No. 344057)
Sorvall HB-4 or HB6 rotor
Polyallomer tube (Sarstedt)
Syringes
Needles ∅ 1.2 mm

- Collect eggs and keep in separate batches if possible
- Wash eggs in 1 X MMR
- Dejelly 2 x 2.5 min with Dejelly Buffer
- Wash 2 x with XB-CSF
- Wash 1 x with XB-CSF + protease inhibitors
- Preload Beckmann tube with 1 ml XB-CSF + 1 µl Cytochalasin B
- pack eggs (place Beckmann tube into polyallomer tube, spin 500 rpm for 30 s, then 2000 rpm for 1.5 min at 14°C)
- spin (rotor HB4 / HB6 RT!) in cold (4°C) centrifuge for 20 min at 10 700 rpm
- collect cytoplasmic fraction with syringe (∅ 1.2 mm)
- add cytochalasin B, final conc. 10 µg/ml
- add protease inhibitors
- (spin extract for add. 60 min at 50 000 rpm in TLS55, collect supernatant as high speed extract)

T**esting the Extract**

labeled tubulin
any kind of nucleating source (sperm nuclei, centrosomes, RanGTP, TPX2,...)

fix
coverslides
coverslips

Fix
0.3 ml formaldehyde 37%
0.6 ml glycerol 80%
1 ml 1x MMR
1 µl Hoechst No. 33342 (10 mg/ml)

100 µl aliquots
keep at -20ºC

test 20 µl extract for CSF-arrest by adding

- labeled tubulin at 1/50 – 1/200 (depending on labeling ration)
- nucleating source
- incubate the extract for 20 min at 16-20ºC
- fix 3-4 µl reaction by squash fixing with 3-4 µl fix solution under a coverslip
- check for mitotic structures

Interphase Extract

in case you need interphase extract, add

- 0.6 mM $CaCl_2$
- Cycloheximide, final conc. 100 µg/ml
- incubate for 40 min at 16-20ºC
- check for interphasic structures as described above