

Preparing CSF Extract

*Kirschner Laboratory
Harvard Medical School
Boston, MA 02115*

Prime Frogs

40-50 frogs primed with PMSG (500 units)
5-7 days later, primed again with 0.5 ml HCG (500 units)
Transfer 2 frogs to each bucket containing 4L of 1X MMR (160 ml 25X MMR + 3840ml frog water)
Leave buckets @ 16°C overnight

Egg Collection

~ 40 Frogs yield 1 – 1.2L eggs
Collect Eggs 24 hours after HCG injection
Remove and discard “strings” with forceps
Throw away entire bucket if more than 10% of eggs are dead.

To Dejelley

Add ~1600ml of 2% cysteine to laid eggs
Incubate at room temperature for 10-15 minutes until the eggs are tightly packed (overall volume should reduce to 1/5 original size).
Wash once with remaining 400ml of 2% cysteine.
Wash 3-6 times with XB buffer, gently pour off dead eggs each time.
Wash 3 times with CSF-XB buffer, manually pick out and discard dead eggs.
Pour off CSF-XB buffer until only 50ml remains on top of eggs.
Add 100 µl PI stock to the eggs, mix well, and incubate at room temperature.
Add 3ml of CSF-XB to each of the SW28 centrifuge tubes.
Add 30 µl cytochalasin B and 3 µl PI stock to each tube.
Transfer ~30 ml eggs to each centrifuge tube.
Suck excess CSF-XB from the top of each tube.
Add ~ 1-2ml of Versilube oil on top of eggs in each tube.

Egg Crushing

Transfer tubes to a HB- 6 rotor with ADAPTORS IN PLACE.
Spin @ 1300 rpm for 1 minute. Remove excess solution on top of egg.
Spin @ 10,000 rpm for 10 minutes at 4 °C
Collect supernatant by side punctuation of the tube.
Add 10 µl of cytochalasin B and 10 µl of PIs to each of the 10ml extracts.
Transfer to a new centrifuge tube and spin at 10,000 rpm, for 10 minutes at 4 °C.
Collect supernatant and add 1.69ml sucrose and 635ml energy mix to 10 ml extract, freeze with liquid nitrogen.