

Methods



CSF-EXTRACT(EPPIE)

Initials JS

Reference

Recipes used in protocol	Amount Used
CSF-XB	
Cytochalasin	
Energy mix, 35x	
MMR, 1x	

Protocol

- 1) Collect eggs in MMR.
- 2) Wash eggs several times in MMR. Dejelly in 2% cysteine in MMR. DO NOT OVERDO. This will cause your eggs to spontaneously activate. Wash 5-6 times in 1xMMR.
- 3) Wash eggs in CSF-XB, preferably at 16-19C. Pour off most of the buffer and add an equal volume of CSF-XB containing 20 ug/ml cytochalasin. Incubate for 10 minutes.
- 4) Place the eggs in tubes containing 1/10th volume CSF-XB with 100 ug/ml cytochalasin B, and 10 x LPC. Use 2 ml tubes.
- 5) Pack the eggs for 30 s at 30 g. Aspirate buffer that rises to the top, and any puffballs (eggs that have lysed within the vitelline envelope). Good eggs will pack uniformly and not lyse. The eggs should orient so that the pigmented animal poles are up, and the vegetal poles are down.
- 6) Crush the eggs at 21,000 × g in 4C microcentrifuge using the fixed angle rotor for 5 min. The cytoplasmic layer was removed (Try to avoid the membrane layer at the bottom!)and spun twice more at 21,000 × g.
- 7) Add energy mix, LPC (1000x stock), and cytochalasin (10ug/ml final). The extract is now ready to use. Nuclei added to the extract should begin to show signs of mitotic condensation within 20 minutes at RT.