Preparation of deyolked embryos

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Zebrafish embryos (500 – 2000) in 3.5 cm dish

↓ reduce the culture medium to about 1 mL

↓ add 1 mL Pronase stock

↓ 28.5°C, 15 min, in incubator

↓ wash with ice cold E3 medium, thoroughly

↓ transfer to another dish with blue tip (for de-chorion)

↓ wash with ice cold deyolking buffer, 3 times

↓ deyolk by pipetting with yellow tip (check with microscope)

↓ transfer to Eppen tube(s) (two tubes for >1000 embryos)

↓ fill up with ice cold deyolking buffer

↓ mix by shaker (EYELA, CM-1000), 5 min, speed 8

↓ cfg., 400 x g, 4°C, 30 sec

ppt.

↓ suspend in 1.5 mL ice cold wash buffer

↓ mix by shaker, 5 min, speed 8

↓ cfg., 400 x g, 4°C, 30 sec

ppt.

↓ suspend in 1.5 mL ice cold PBS

↓ mix by shaker, 5 min, speed 8

↓ cfg., 400 x g, 4°C, 30 sec

ppt.

↓ store at -70°C

➢ Pronase stock (for zebrafish embryo)

・Stock solution

5 mg/mL Pronase, 0.05% Phenol red in E3 medium

・Preparation

10 mg Phenol red

↓ 20 mL E3 medium

↓ add several drops of 1 mM $NaHCO_3$ to turn red color

↓ dissolve 100 mg Pronase

↓ aliquot each 1 mL in Eppen tubes

↓ store at -20°C