

## *D* *evelopment of primary cell cultures from Ciona intestinalis larval stage*

1. Gametes from adult *Ciona intestinalis* were used for *in vitro* fertilization and resulting embryos were cultured until the larval stage at 16-18°C in filtered sea water (FSW) containing penicillin and streptomycin.
2. Embryos at larval stage were collected by hand centrifugation (pellet ~30 µl) and dissociated in 1 ml of sterile artificial sea water (ASW) supplemented with collagenase P, trypsin and DNaseI, for 30 min at room temperature.
3. Embryos were triturated with a sterile glass pipette and cell suspension was diluted 10 times with ASW to block enzyme activity and filtered through a 40 µm nylon membrane.
4. Digestion solution was removed and the pellet resuspended in 2 ml of culture medium L15 supplemented with L-glutamine, antibiotics and antimycotics, with or without 10% FBS.
5. Cells were seeded into 35-mm plastic plates or onto glass cover-slips coated with poly-L-lysine.
6. Culture medium was added up to 2 ml and plates were incubated at 16-18°C.
7. Culture medium was renewed every 2 days and cell debris were removed by rinsing once with serum-free medium.

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*Ciona intestinalis*: adult specimens  
collected in the Bay of Naples.

Cell culture apparatus: cooled incubator  
and biological safety cabinet class II.

Cell culture medium: Leibovitz's medium  
(L15) adjusted to sea water salt  
concentration.

Medium supplements: Fetal bovine serum  
(FBS), 0.3 mg/ml L-glutamine, 100 U/ml  
penicillin, 0.1 mg/ml streptomycin and  
0.25 mg/ml amphoterycin.

Solutions: embryo culture solution  
(20,000 U/ml penicillin and 50 mg/ml  
streptomycin in filtered sea water, FSW),  
artificial sea water (ASW: 460 mM NaCl,  
10 mM KCl, 55 mM MgCl<sub>2</sub>, 11 mM CaCl<sub>2</sub>,  
10 mM glucose, 10 mM HEPES), digestion  
solution (1 mg/ml collagenase P,  
1 mg/ml trypsin, 0.1% DNaseI in ASW),  
coating solution (0.1 mg/ml poly-L-lysine  
in 0.1M sodium borate pH 8.2).

Plasticware: cell culture dishes and  
serologic pipettes from Corning.

All chemicals were purchased from  
Sigma-Aldrich.