Primary cell cultures from total Paracentrotus lividus *coelomocytes*

1. Withdraw coelomic fluid (approx. 25 ml) from adult specimens of sea urchin *Paracentrotus lividus* through a cut in the peristomial membrane using a syringue containing 25 ml of Tris-EDTA to prevent clotting.
2. Centrifuged collected coelomic fluid for 5 min at 1000×g at RT.
3. Discard the supernatant and suspend the cell pellet in 25 ml of microfiltered coelomic fluid (MCF).
4. Cells are seeded in 24-well plates at a density of 10^5 cells per well and cultured with 1 ml of MCF.
5. Replace progressively MCF by cell culture medium (CCM), removing twice per week 500 µl of liquid and replacing it by fresh CCM.

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Sea urchin: Adult specimens of sea urchin Paracentrotus lividus collected in the bay of Banyuls, 4-5 cm in diameter, maintained in aquarium at 18°C with quality controlled sea water.

Apparatus: A cooled cell incubator; a class II biological safety cabinet.

MCF: coelomic fluid collected and micro-filtered on 0.2 µm membrane (Millipore).

CCM: Cell culture medium composed of NaCl 0.5 M, $MgCl_2$ 5 mM, EGTA 1 mM, HEPES 20 mM, pH 7.2 (Henson et al., 1999) supplemented with 10% L-15 medium (Sigma L4386), 5% foetal bovine serum (GIBCO 10270) and penicillin 100 units, streptomycin 0.1 mg/ml.

Solution: Tris-EDTA: Tris-HCl 40 mM pH 7.5, EDTA 1.4 M, NaCl 1M).

Plasticware: 24-well cell culture dishes from Corning.

All chemicals were purchased from Sigma-Aldrich.

Additional information:

***Henson JH, Svitkina TM, Burns AR, Hughes HE, MacPartland KJ et al.** (1999) Two components of actin-based retrograde flow in sea urchin coelomocytes. Mol Biol Cell 10:4075-4090*

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