

*P*reparation of Ascidian hemolymph for the culture of *Ciona* cells

PREAMBULE. Live ascidians must be handled with care to avoid body contraction.

1. Using a scalpel, cut the body wall (tunic and muscle layer) on the left side and at the bottom end of animals, in order to expose the visceral apparatus. The gonad is surrounded by the intestinal loop. The heart is behind as a transparent tube bent into a V shape. Blood movements are visible inside unbroken hearts.
2. Using tweezers, carefully draw out the heart and lay it on the rim of a micro-centrifuge tube, making it jutting inside the tube.
3. Prick it with a needle and wait for some seconds in order to collect the hemolymph.
4. Estimate the amount of hemolymph obtained and eventually proceed with another animal.

Alternative: in transparent ascidians it is possible to draw the hemolymph by suction using a syringe, going into the pericardic cavity (close to the heart), trough the right side of the body wall.

5. Pool the hemolymph collected from various animals and centrifuge at 3000 rpm for 5 min at RT.
6. Collect the supernatant and filter-sterilize using 0.2 μm filters; store aliquots (200 μl) at -20°C .

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Animals: healthy adult specimens of *Ciona intestinalis* (body length approx. 10-15 cm) collected in the Bay of Naples.

Apparatus: centrifuge

Materials: scalpel, tweezers, needles, microcentrifuge tubes, ice bath.

ASSEMBLE

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