



Incubation Instruction with Decapsulation

1. Prepare incubation solution by using NaCl. Concentration (salinity) has to be 28-30 ppt. You can use sea salt. Alternatively, you can use prepared (preferably UV or Ozone) seawater as well.
2. pH of the prepared solution has to be 7,9-8,5. If the pH is lower – add 2 grams of Sodium Bicarbonate (NaHCO_3) per each 1 Liter of the solution.
3. Temperature of the incubation solution has to be 27 – 29 °C.
4. Prepare decapsulation solution. For 454 g of cysts you need 1,5L of NaOCl and 34-50 ml of NaOH saturated solution.
Ratio: 1L of liquid bleach (NaOCl with 10% of Cl) with additional 23-33 ml of NaOH saturated solution.
5. Hydration. Put the cysts into fresh water, with strong aeration for 1 hour.
6. Separate the hydrated cysts from the water (the shape of an egg has to change from biconcave to spherical).
7. Put the hydrated cysts into the decapsulation solution. Volume of the solution will be half of the prepared volume, or 0,75 L. Keep stirring the cysts and the solution constantly for 25-40 minutes. Because of this an exothermic reaction will occur, if the temperature rises up – cool the reservoir by hosing the outer sides of the tank with cold water.
8. Separate the cysts from the decapsulation solution by 100-microns net. Wash the cysts with running water (clean seawater).
9. Repeat Step 7 for 40-50 minutes (note that the duration increased).
10. When the color of the cysts changes from white to orange – separate the cysts from the decapsulation solution and wash them with clean seawater.
11. 30 minutes before the next stage (putting the cysts into incubation solution) – dissolve an activator in the incubation solution (Sodium Erythorbate ($\text{C}_6\text{H}_7\text{O}_6\text{Na}$), 0,45g/1g cysts, or 200 g per 454 g cysts).
12. Put the orange decapsulated cysts in the prepared incubation solution (2 grams of cysts per every 1 Liter of incubation solution).
13. Plug on aeration in the incubation reservoir.
14. Provide 1500-2000 lux of illumination.
15. Incubation process is started. Wait for 24 hours.
16. Plug off aeration. Pour the shells out. Filter nauplii. Washing is optional.
17. Nauplii are ready. Bon Appetit.

Precautions & Warnings:

Try to use a conical reservoir, because of its hydrodynamic features, the result is always better.
1500-2000 Lux – approximately corresponds to 100W glow-lamp, in a distance of 1 meter.
Opened pack has to be kept away from water.

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