

Materials List for:

Preparation of *Aplysia* Sensory-motor Neuronal Cell Cultures

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Materials

Solutions needed for culture

1. 0.35 M MgCl₂, stored at room temperature, used for anesthesia.
2. Poly-L-lysine solution, Sigma: P-1524, MW >300,000, made in 0.1M Sodium Borate pH8.2 to 0.5mg/ml solution. Vortex well and filter sterilize through a 0.22 µm filter, store it at 4C°. Do not freeze-thaw.
3. L-15 Medium powder (Leibovitz) (Sigma: L4386) supplemented with the salts as below to make 1 liter

L-15 powder	13.8g
NaCl	15.4 g
D-Glucose	6.24 g
MgSO ₄ ·7H ₂ O	6.45g
KCl	350 mg
NaHCO ₃	170 mg
MgCl ₂ ·6H ₂ O	5.49 g
CaCl ₂ ·2H ₂ O	1.43g
HEPES	3.53g

Add ddH₂O to 1 liter. The pH should be about 7.4-7.5, add 10ml of 100X Pen/Strep solution, and filter-sterilize through a 0.22 µm filter. Store at 4C0 for no longer than 1 month.

4. Protease digestion solution: 1% Protease IX (1unit/mg) is made in L15 (supplemented with salts as above) or in ASW immediately before use, filter-sterilized through a 0.22 µm Millipore. 5mls should be enough for the ganglia from two animals (make sure the ganglia are completely immersed in protease solution). Sigma has reported that they will discontinue selling Protease IX. A substitute protease is: Dispase II (Roche Applied Science catalog # 04942078001).
5. Culture medium. Immediately prior to preparing cultures, thaw a 10 ml aliquot of hemolymph and mix it with 10 mls of L15 (supplemented with salts as above) to make 20mls culture medium. Add 200 µl of 200mM L- Glutamine, mix well and use for preparing cultures. This medium should be prepared fresh each time cultures are made.
6. Artificial Seawater can be made from Instant Ocean (Aquarium Systems, Mentor, OH) or as follows: 450 mM NaCl, 10 mM KCl, 30 mM MgCl₂(6H₂O), 20 mM MgSO₄, 10 mM CaCl₂(2H₂O), 10 mM HEPES, with pH adjusted to 7.4.