

Solutions for Sea Urchin Development

ASW: Artificial sea water (Woods Hole MBL)

NaCl	24.72 g	
KCl	0.67 g	
CaCl ₂ .2H ₂ O	1.36 g	
MgCl ₂ .6H ₂ O	4.66 g	
MgSO ₄ .7H ₂ O	6.29 g	
NaHCO ₃	0.18 g	
dH ₂ O	to 1 liter	<u>make 5 liters</u>

Filter 1 L through 0.2 µm filter unit into sterile bottle and add antibiotics:
200 mg/L sulfamethoxazole and 10 mg/L trimethoprim

0.5 M KCl to induce gamete shedding

3.73 g KCl/ 100 ml dH₂O

Urchins

S. purpuratus (late Fall and Spring) and *L. pictus* (early Fall) can be ordered from Marinus Scientific (714-901-9700). Note: these are Pacific (cold water) urchins and California collecting licence is needed to obtain these urchins.

L. variegatus (Spring) can be ordered from Carolina Biologicals (800-335-5551).

Other supplies

Sulfamethoxazole, Sigma S-7507

Trimethoprim, Sigma T-7883

Prep checklist:

For preparation of gametes:

- 25 g needles
- 1 ml or 3 ml syringes
- needle disposal container
- aluminum foil
- 100 mm petri dishes
- Controlled drop pasteur pipets (Fisher 13-678-30)
- Pipet bulbs
- Clean 100 ml beakers (never washed with soap), sterile
- ASW , 0.2 μ m filtered with antibiotics
- 0.5 M KCl
- Cheesecloth and 600 ml soap-free beakers
- Microfuge tubes (to store sperm)
- T75 tissue culture flasks (to store eggs from *S. purpuratus* and *L. pictus*)

For each table:

- test tube rack
- marker
- labelling tape
- Paper towels (C-fold)
- 100 mm tubes (Fisher 14-961-27, never washed)
- Controlled-drop pasteur pipets and bulbs
- depression slides
- microscope slides
- coverslips
- Gloves- small, medium and large (1 box each)
- ASW

Equipment:

- Chilled water bath or e-ICE bucket at 15°C (for *S. purpuratus* and *L. pictus*)
- refrigerator
- compound microscopes
- incubator at 15°C (for *S. purpuratus* and *L. pictus*)