

# Water

(Source: M. Westerfield from [Zebrafish Book 5th Edition](#))

Several different types of water are used for procedures described in the following sections. These solutions have been adapted to balance ease and cost of production with the changing needs of zebrafish during their life cycle.

In general, adults can be maintained in tap water, conditioned by letting it set. This depends critically on the quality of the local water source and on the demands for embryo production. Poor water quality will adversely affect the health of the fish, their susceptibility to disease, and their breeding potential. If there is any question about water quality, deionized or distilled water should be used to which a small amount of salts and minerals are added. If an adequate supply of deionized water is unavailable, a closed system that recirculates the water after purification can be used.

Embryos and young larvae have stricter requirements and should be raised in egg water. Embryos removed from their chorions require additional calcium and should be maintained in embryo medium.

*System (or tank) water:* Water out of the facility's water system. This water is dripped into clean tanks and is used for setting up pairwise crosses.

*Egg water:* Used for *in vitro* fertilization and raising young embryos.

**Stock salts:** 40 g "Instant Ocean®" Sea Salts added to 1 L distilled water

**Egg water:** 1.5 ml stock salts added to 1 L distilled water = 60g/ml final concentration.

**Embryo medium:** Do not confuse with "egg water" above. Use this medium for handling dechorionated embryos and storing young embryos in dishes. This is basically 10% Hank's with full strength calcium and magnesium.

**Embryo medium:** 1.0 ml Hank's Stock #1, 0.1 ml Hank's Stock #2, 1.0 ml Hank's Stock #4, 95.9 ml ddH<sub>2</sub>O, 1.0 ml Hank's Stock #5, 1.0 ml fresh Hank's Stock #6. Use about 10 drops 1 M NaOH to pH 7.2

**Hank's solutions:** Hank's solutions can be made from stock solutions (kept refrigerated, they will last for several months). A premix of the salts can be stored in the refrigerator for several weeks. Sodium bicarbonate does not store well, so it is made up fresh each time Hank's solution is made.

**Full Strength Hank's:** 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.44 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM CaCl<sub>2</sub>, 1.0 mM MgSO<sub>4</sub>, 4.2 mM NaHCO<sub>3</sub>

## **Hank's Stock Solutions:**

Stock #1 8.0 g NaCl 0.4 g KCl in 100 ml ddH<sub>2</sub>O

Stock #2 0.358 g Na<sub>2</sub>HPO<sub>4</sub> Anhydrous, 0.600 g KH<sub>2</sub>PO<sub>4</sub>, in 100 ml ddH<sub>2</sub>O

Stock #4 0.72 g CaCl<sub>2</sub> in 50 ml H<sub>2</sub>O

Stock #5 1.23 g MgSO<sub>4</sub>·7H<sub>2</sub>O in 50 ml ddH<sub>2</sub>O

Stock #6 0.35 g NaHCO<sub>3</sub> 10.0 ml ddH<sub>2</sub>O

## **Hank's Premix:**

Combine the following in order:

10.0 ml Solution #1, 1.0 ml Solution #2, 1.0 ml Solution #4, 86.0 ml ddH<sub>2</sub>O, 1.0 ml Solution #5

Store Hank's Premix in the refrigerator along with the Hank's solutions.

## **Final Hank's:**

9.9 ml Hank's Premix, 0.1 ml fresh Stock #6