

## E2 Embryo Media with Methylene Blue (Revised May 2013 JLM)

At ZIRC 0.5X E2 with 0.5 mg/L Methylene Blue is used as a working solution (Conductivity $\approx$ 1000-1100  $\mu$ S; Osmolality $\approx$ 28 mmol/kg). We prepare three concentrated stock solutions, E2A, E2B and E2C, and 0.1% Methylene Blue which are then used to make a large volume (20L) of the 0.5X E2 working solution. This protocol is a modification (half strength) of the E2 Embryo Medium described in C. Nüsslein-Volhard and R. Dahm (2002) ZEBRAFISH, Oxford University Press, A Practical Approach.

---

E2A (100X) contains:

1.5	M	NaCl
50	mM	KCl
100	mM	MgSO <sub>4</sub>
15	mM	KH <sub>2</sub> PO <sub>4</sub>
5	mM	Na <sub>2</sub> HPO <sub>4</sub>

To prepare 2 L of E2A (100X) combine the following according to the instructions below:

**175.0 g Sodium Chloride (NaCl)**  
**7.5 g Potassium Chloride (KCl)**  
**49.3 g Magnesium Sulfate Hepahydrate (MgSO<sub>4</sub>-7H<sub>2</sub>O) {Can substitute 24.1 g of Anhydrous MgSO<sub>4</sub>}**  
**40 mL E2A Buffer Mix**

- In a 2 L beaker, add a large stir bar and Nanopure dH<sub>2</sub>O to 1600 ml
  - Add the above dry reagents in the order listed; stir to dissolve each one completely before adding the next reagent
  - Add 40 mL E2A Buffer Mix (see recipe below), continue stirring to mix
  - Transfer solution to a 2 L graduated cylinder and adjust final volume to 2.0 L with Nanopure dH<sub>2</sub>O
  - Cover the graduated cylinder with Parafilm and invert 2-3 times to mix completely
  - Filter sterilize into two 1 L bottles
  - Store in refrigerator at 4°C
- 

E2A Buffer Mix contains:

750	mM	KH <sub>2</sub> PO <sub>4</sub>
250	mM	Na <sub>2</sub> HPO <sub>4</sub>

To prepare 1L of E2A Buffer Mix combine the following according to the instructions below:

**102.1 g Potassium Phosphate Monobasic (KH<sub>2</sub>PO<sub>4</sub>)**  
**67.0 g Sodium Phosphate Dibasic Heptahydrate (Na<sub>2</sub>HPO<sub>4</sub>-7H<sub>2</sub>O) {Can substitute 35.5 g of Anhydrous Na<sub>2</sub>HPO<sub>4</sub>}**

- In a 1 L beaker, add a large stir bar and Nanopure dH<sub>2</sub>O to 750 mL
  - Add the above dry reagents in the order listed; stir to dissolve each one completely before adding the next reagent
  - Transfer solution to a 1 L graduated cylinder and adjust final volume to 1.0 L with Nanopure dH<sub>2</sub>O
  - Cover the graduated cylinder with Parafilm and invert 2-3 times to mix completely
  - Filter sterilize
  - Store in refrigerator at 4°C
- 

E2B (500X) contains:

500	mM	CaCl <sub>2</sub>
-----	----	-------------------

To prepare 1 L of E2B (500X ) combine the following according to the instructions below:

**73.5 g Calcium Chloride Dihydrate (CaCl<sub>2</sub>-2H<sub>2</sub>O)**

- In a 1 L beaker, add a large stir bar and Nanopure dH<sub>2</sub>O to 800 mL
  - Add the above dry reagent and stir to dissolve completely
  - Transfer solution to a 1 L graduated cylinder and adjust final volume to 1.0 L with Nanopure dH<sub>2</sub>O
  - Cover the graduated cylinder with Parafilm and invert 2-3 times to mix completely
  - Filter sterilize
  - Store in refrigerator at 4°C
-

---

**E2C (500X)** contains: 350 mM NaHCO<sub>3</sub>

To prepare 500 mL of E2C (500X ) combine the following according to the instructions below:

**14.7 g Sodium Bicarbonate (NaHCO<sub>3</sub>)**

- In a 1L beaker, add a medium stir bar and Nanopure dH<sub>2</sub>O to 350 mL
- Add the above dry reagent and stir to dissolve completely
- Transfer solution to a 500 mL graduated cylinder and adjust final volume to 500 mL with Nanopure dH<sub>2</sub>O
- Cover the graduated cylinder with Parafilm and invert 2-3 times to mix completely
- Filter sterilize
- Aliquot into 20 ml portions (in 50 ml Falcon tubes)
- Store in lab freezer at -20°C
- Thaw 20 mL aliquot just prior to use

---

**0.1% Methylene Blue**

To prepare 1L of 0.1% Methylene Blue, combine the following according to the instructions below:

**1.0 g Methylene Blue**

- Add 1.0 L Nanopure dH<sub>2</sub>O to a 1 L bottle
- Add the above dry reagent to the bottle and shake to dissolve completely
- Store at room temperature (28°C)

---

**FINAL WORKING SOLUTION: 0.5X E2 with Methylene Blue:**

To prepare 20L of 0.5X E2 with Methylene Blue

- Fill 20 L carboy with Reverse Osmosis water to 19 L, aerate until ready to mix
- Add the following to the carboy in the order listed (shake/stir all solutions before measuring)

**100 mL 100x E2A**  
**20 mL 500x E2B**  
**20 mL 500x E2C**  
**10 mL 0.1% Methylene Blue**

- Adjust final volume to 20 L with Reverse Osmosis water
- Aerate and stir to mix
- Flush carboy spout by flowing out 200-400 mL and pouring back into top of carboy
- Remove a small aliquot (~50 mL) to check conductivity and pH
- Adjust pH to 7.2-7.6 (with concentrated HCl or concentrated NaOH) if necessary
- Store and use at room temperature (28°C), make fresh weekly

**E2 Embryo Media Final Working Solution Molar Concentrations:**

**0.5X E2**

7.5	mM	NaCl
0.25	mM	KCl
0.5	mM	MgSO <sub>4</sub>
75	µM	KH <sub>2</sub> PO <sub>4</sub>
25	µM	Na <sub>2</sub> HPO <sub>4</sub>
0.5	mM	CaCl <sub>2</sub>
0.35	mM	NaHCO <sub>3</sub>
0.5	mg/L	Methylene Blue

**1.0X E2**

15	mM	NaCl
0.5	mM	KCl
1.0	mM	MgSO <sub>4</sub>
150	µM	KH <sub>2</sub> PO <sub>4</sub>
50	µM	Na <sub>2</sub> HPO <sub>4</sub>
1.0	mM	CaCl <sub>2</sub>
0.7	mM	NaHCO <sub>3</sub>
0.5	mg/L	Methylene Blue