

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

EMBRYO MEDIA E2:

The 1X E2 medium contains:

15.0	mM	NaCl
0.5	mM	KCl
1.0	mM	MgSO ₄
0.15	mM	KH ₂ PO ₄
0.05	mM	Na ₂ HPO ₄
1.0	mM	CaCl ₂
0.7	mM	NaHCO ₃

At ZIRC, **0.5X** E2 is used as a working solution.

Typically, we prepare three stock solutions, called E2A, E2B and E2C, which are then used to make a large volume of the 0.5X E2.

E2A:

We make 100X E2A by dissolving the following ingredients in a final volume of 1600 ml:

- 140.0g NaCl
- 6.0g KCl
- 19.2g MgSO₄
- 3.3g KH₂PO₄
- 1.1g Na₂HPO₄

- add millipore water to 1600 ml
- shake and stir to dissolve the reagents
- autoclave
- stir O/N to dissolve any precipitation that has formed during autoclaving
- store at 4°C

E2B:

We make 500X E2B by dissolving 11.0 g CaCl₂ (or 14.6g CaCl₂ x 2H₂O) in the final volume of 200 ml

- add millipore water to 200 ml
- shake to dissolve the reagent
- autoclave
- aliquote into 20 ml portions (in 50 ml Falcon tubes)
- store in -20°C

E2C:

We make 500X E2C by dissolving 6.0g NaHCO₃ in the final volume of 200 ml

- add millipore water to 200 ml
- shake to dissolve the reagent
- autoclave
- aliquote into 20 ml portions (in 50 ml Falcon tubes)
- store in -20°C

TO MAKE 20 LITERS 0.5X E2, mix:

100 ml 100x **E2A**
20 ml 500x **E2B**
20 ml 500x **E2C**

- add Reverse Osmosis water to 19 liters
- adjust pH to 7.0-7.5 (with concentrated HCl or concentrated NaOH)
- add Reverse Osmosis water to 20 liters
- store at room temperature