

Formulations for physiological salines for amphibian research

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Physiological salines are solutions are formulated to simulate the ionicity – concentration of salts - of various blood and other plasmas, lymphatic fluid, and cellular exudations. In amphibian research they are used for a variety of purposes including standardized aquatic media for oocyte storage, egg incubation and larval rearing, and as bases for cryodiluents. Stock solutions can be prepared and then diluted for use. If stored for any time they should be refrigerated. In veterinary practice moribund amphibians can be placed in tubs with physiological salines to replenish salt loss and reestablish hydration.

Simplified Amphibian Ringers

	Sigma	MWt	g l ⁻¹	For 3 litres
113.0 mM NaCl	S 5886	58.44	6.600 g	19.8
1.0 mM CaCl ₂	C 7902	147.00	0.147 g	0.441
2.0 mM KCl	P 5405	74.55	0.149 g	0.447
3.6 mM NaHCO ₃	S 5761	84.01	0.302 g	0.906

Modified Amphibian Ringer

	Sigma	MWt	g l ⁻¹	For 3 litres
100.0 mM NaCl	S 5886	58.44	5.840 g	17.52 g
1.8 mM KCl	P 5405	74.55	0.134 g	0.402 g
1.0 mM MgCl ₂	M 2393	203.30	0.203 g	0.609 g
2.0 mM CaCl ₂	C 7902	147.00	0.294 g	0.882 g
5.0 mM Na-Hepes	H 3784	260.30	1.300 g	3.900 g

Phosphate buffered saline PBS

A 10 liter stock of 10x PBS can be prepared by dissolving 800 g NaCl, 20 g KCl, 144 g Na₂HPO₄ · 2H₂O and 24 g KH₂PO₄ in 8 L of distilled water, and topping up to 10 L. The pH is ~6.8, but when diluted to 1x PBS it should change to 7.4. When making buffer solutions, it is good practice to always measure the pH directly using a pH meter. If necessary, pH can be adjusted using hydrochloric acid or sodium hydroxide.

On dilution, the resultant 1x PBS should have a final concentration of 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH of 7.4.

Holtfreter's balanced salt solution

350 mg NaCl

0.5 mg KCl

0.01 mg. CaCl₂ per liter

Calcium Free Saline (for cell dissociation with collagenase)

	Sigma	MWt	g 500ml ⁻¹
82.5 mM NaCl	S 5886	58.44	2.40
2.5 mM KCl	P 5405	74.55	0.093
1 mM MgCl ₂	M 2393	203.30	0.10
5mM Hepes (pH 7.6)	H 3784	260.3	0.65
Collagenase A	C 0130	NA	2mg/ml

DeBoer's solution DB2

110 mM NaCl

1.3 mM KCl

0.44 mM CaCl₂

pH 7.3

Phosphate buffered water

Phosphate buffer usually is buffered to pH 7.2. A convenient site for the calculation of the amounts of compounds for phosphate buffers of different pH and strength may be found at <http://members.nuvox.net/~on.jwclymer/phos2.html>.

Or a simple formula is (as table below) for a buffer of pH 7.2 is 35ml of 0.2M NaOH plus 50ml of 0.2M NaH_2PO_4 .

0.2M NaOH = (MW= 40) therefore 0.2M is 8g per. liter.

0.2M KH_2PO_4 = (MW = 120) therefore 0.2 M is 25g per. liter.

This table shows the volume (ml) of 0.2M NaOH solution to be added to 50ml of 0.2M KH_2PO_4 to achieve a particular pH.

pH	NaOH (ml)	pH	NaOH (ml)
5.8	3.7	7.0	29.6
6.0	5.7	7.2	35.0
6.2	8.6	7.4	39.5
6.4	12.6	7.6	42.8
6.6	17.8	7.8	45.2
6.8	23.7	8.0	46.8

Marc's Modified Ringer, Xenopus Care, <http://www.xlaevis.com/> info@xlaevis.com

4 Liters 1xMMR (Marc's Modified Ringers)

NaCl	23.10g
CaCl ₂ (2H ₂ O)	1.28 g
MgCl ₂ (2H ₂ O)	0.99 g
KCl	0.60 g
Hepes	4.76 g
EDTA	0.27 g

Add distilled water to 4 liters. pH to 7.4

Dilute by a factor of ten to make 0.1 x MMR