

Rat Sperm Thawing Protocol

Rat Resource and Research Center

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Abbreviations:

R1CH: R1CC-HEPES, a modified R1CC where part of bicarbonate is replaced with HEPES buffer.

1.1. Rat stock solution:

1. To the appropriate sized Griffin Beaker containing Embryo Transfer water (Sigma; W1503; 80% of the total volume of media), add the components in grams according to **Worksheet 1.1**.
2. After all components have dissolved, transfer the contents to the appropriate volumetric flask and bring to desired volume by rinsing out the Griffin beaker with Embryo Transfer water and adding it to the volumetric flask. Measure the osmolality, record this result, and filter into an appropriately sized sterile bottle.
3. Label with name of solution, date made, expiration date (one month from date made) and initials. Label with osmolality and batch number. Store at 4°C.

Worksheet 1.1: Rat stock solution (10X stock)

To the appropriate sized Griffin Beaker containing Embryo Transfer water (Sigma W1503, 80% of the total volume of medium), add the following components in grams:

Company	Cat #	Reagent	FW (g)	mM (Working)	500 ml	Added	Lot#
Sigma	S5886	NaCl	58.44	80	23.376g		
Sigma	P5405	KCl	74.55	3.2	1.1928g		
Sigma	G6152	D-Glucose	180.2	7.5	6.7576g		
Sigma	P7794	Penicillin G K Salt	372.2	100µg/ml	0.375g		
Sigma	S1277	Streptomycin Sulfate	1457	50µg/ml	0.25g		
Sigma	L7900	Sodium Lactate (60% syrup)	186.8	13.53	12.637g		
Sigma	C7902	CaCl ₂ -2H ₂ O	147.02	2	1.4702g		
Sigma	M2393	MgCl ₂ -6H ₂ O	203.31	0.5	0.5083g		
Osmolality							

Lot# for Embryo Transfer water	
Batch (lot)#	
Your initials:	
Today's date	
Expiry date 1 month from preparation date	

1.2. Protocol for making R1CH:

1. Add all components as listed in **Worksheet 1.2** (next page) to a Griffin beaker containing Embryo Transfer water (80% of final volume).
2. After all components have been dissolved, check pH and adjust pH to ~7.4 with 1N NaOH.
3. Transfer the contents to the appropriate volumetric flask and bring to desired volume by rinsing out the Griffin beaker with Embryo Transfer water and add it to the volumetric flask.
4. After calibrating the osmometer with a 290 mOsm standard, check the osmolality of the solution. Remake the solution if the osmolality does not fall between **280-300 mOsm**.
5. Sterile filter the solution using a 0.22 μm filter unit.
6. Label container with date made, expiration date (four weeks from date made), pH, mOsm, your initials and batch number.
7. Store at 4°C for up to 4 weeks.

Note: If any precipitates form in the solution, discard the solution and start over.

A. Working solution:

1. Aliquot amount needed (usually 10 ml) using a sterile pipette.
2. Add 4 mg/ml (0.04 g/10 ml) Fatty Acid Free BSA (Sigma A7638).
3. Sterile filter using 0.22 μm syringe filter.

Worksheet 1.2. R1CH (high NaCl)

- Record Embryo Transfer water used: Lot #:
- Add the following components to Griffin Beaker containing Embryo Transfer water.

Catalog #	Reagent	Final Conc (mM)	g/500 ml	Added	Lot Number
See stock solutions	Rat Stock	n/a	50 ml		
Sigma S5886	NaCl	30	0.8766		
Sigma S5761	NaHCO ₃	5	0.2100		
Sigma P4562	Sodium Pyruvate	0.5	0.0276		
Invitrogen 11140-050	MEM NEAA 100x	n/a	5 ml		
Invitrogen 11130-051	MEM EAA 50X	n/a	10 ml		
Sigma H6147	HEPES	22	2.6214		
Invitrogen 35050-061	GlutaMAX 1	n/a	0.250 ml		

Parameter	Expected	Y or N/ Measured
pH of solution*	7.4	
Osmolality	290-320	
Filter Sterilized	N/A	
Expires	4 weeks from date made	DATE:

Initial pH	
Final pH	
Vol/pH Reagent Added	

*Adjust pH if needed.

Today's date: _____

Your initials: _____

Batch # _____

Please note that this thawing protocol is appropriate for sperm frozen using the method described in the RRRC protocol: Rat Sperm Freezing Protocol at RRRC, 05/2019

Thawing:

1. Remove straw from LN₂ and expose to room temperature for 15 seconds. Then place the straw in a 37⁰C bead bath until the sample is thawed.
2. Cut both ends of straw (end opposite label first) and expel sperm slowly into a 1.5 mL microcentrifuge tube containing pre-warmed (37⁰C) R1CH + 4 mg/mL BSA.

Sperm Analysis

Sperm analysis can be performed using a hemocytometer. At the RRRC, we use a Hamilton Thorne Sperm Analysis Protocol for evaluating sperm *concentration*, *morphology*, and *motility*. (Protocol available upon request).