

6/8/20 NXR

NXR Sperm Freezing, Thawing, and Storage

Cryoprotectant

0.4M sucrose.....	136.92g	}	40ml
10mM NaHCO ₃	0.84g		
2mM pentoxifylline.....	0.56g		
	1000ml		
Vacuum filter			
			+
Organic Egg Yolk.....	~17.5ml	} 1:1	10ml
MQ H ₂ O.....	~17.5ml		
	35ml		
			<hr/>
			50ml

Divide 50ml into 2 x 25ml ultracentrifuge tubes. Spin 20' at 10000 g (Beckman centrifuge, JA-20 rotor) @ 10°C.

Pour supernatant into fresh 50mL falcon, aliquot 1mL and store at -20°C (For at least 2 months)

L-15+calf serum

L-15.....	900µl	
10% calf serum.....	100µl	- Heat inactivated Fetal Bovine Serum store at -20°C
L-glutamine 200mM.....	10µl	
	~1ml	

Make fresh. Put on ice

X.tropicalis

Euthanize a fresh male and extract the testes. Time the procedure to be as efficient as possible, ensure everything is thawed/aliquot before extracting testes.

Use a micropestle to macerate **both testes** from one frog in 1ml ice cold L-15sg in a 1.5ml eppendorf tube. Transfer the sperm/L-15sg mixture into a 2ml tube (or larger) on ice containing 1ml ice-cold cryoprotectant.

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Mix the contents and aliquot 125 μ l each into 16 tubes and transfer them quickly to the back of -80°C freezer in a polystyrene box (~8" x 6" x 5") covered with aluminum foil (No polystyrene lid). Use a stand in the box to keep tubes standing straight. Transfer tubes to liquid nitrogen dewar next day.

X. laevis

Euthanize a fresh male and extract the testes. Time the procedure to be as efficient as possible, ensure everything is thawed/aliquot before extracting testes.

Cut each testis with a scissor, then use forceps to tear the tissue in 1ml ice cold L-15sg in a sterile dish. Transfer the sperm/L-15sg mixture into 2ml tube (or larger) on ice containing 1ml ice-cold cryoprotectant.

Mix the contents and aliquot 125 μ l each into 16 tubes and transfer them quickly to the back of -80°C freezer in a polystyrene box (~8" x 6" x 5") covered with aluminum foil (No polystyrene lid). Use a stand in the box to keep tubes standing straight. Transfer tubes to liquid nitrogen dewar next day.

IVF using frozen sperm:

- Take frozen sperm from LN dewar or -80°C and place in dewar flask with liquid nitrogen or on dry ice
- Obtain medium size clutch (~500) of eggs from 1-2 females.
- Remove frozen sperm from dry ice/liquid nitrogen and immediately swirl in a 37°C water bath for 30s.
- Add ~125 μ l (more can be added for larger cultures) room temperature 0.1x MMR to sperm at 30s.
- Pipet sperm mixture to the clutch of eggs and mix into monolayer.
- After 5-10 min flood the eggs with 0.1 x MMR.

200mM L-glutamine stock:

29.23mg/mL L-glutamine

filter sterilize

aliquot (11 uL aliquots)

store in -20°C until use

Cryosperm aliquots should be stored in liquid nitrogen or at -80°C. If stored at -80°C, aliquots should be placed at the back of the freezer to minimize exposure to temperature fluctuations.