

STOCKS FOR EMBRYO MEDIA

Stock solutions:

HEPES (cell culture grade) 4.766g (10X)
Phenol Red 0.01g
pH to 7.4 with NaOH, bring volume up to 100ml with culture grade dH₂O.

Na bicarbonate (cell culture grade) 2.101g (10X)
bring up to 100 ml with culture grade dH₂O.

Salts: g/100ml (10X)
NaCl 5.146
KCL 0.356
KH₂PO₄ 0.162
MgSO₄ 0.143
glucose 1.0
penicillin G/streptomycin sulfate 5 ml Gibco Pen-Strep
Na lactate 4.085 ml of 60% syrup

Na pyruvate 0.0275 g/10 ml (100X)

Ca lactate·2H₂O 0.385g/10 ml (100X)

FILTER STERILIZE ALL STOCKS AND STORE AT -20°C. BICARBONATE AND Na PYRUVATE STOCKS MAY NEED TO BE REPLACED BEFORE OTHERS. OTHER STOCKS SHOULD BE GOOD FOR 6-12 MONTHS DEPENDING ON THE FREEZER.

EMBRYO MEDIA FROM STOCKS

WORKING MEDIA (50ml) INCUBATION MEDIA (10ml)

1. HEPES	5.0 ml	-----
2. Bicarb	-----	1.0 ml
3. Salts	5.0 ml	1.0 ml
4. Na Pyruvate	0.5 ml	0.1 ml
5. Ca lactate	0.5 ml	0.1 ml
6. dH ₂ O	39 ml	7.8 ml
7. BSA 7	200 mg	40 mg

1. Measure all solutions with plastic pipets--- be accurate.
2. Use culture grade dH₂O.
3. For working media, readjust pH before filtering (pH to 7.3 - 7.4).
4. Filter sterilize and aliquot, store at 4°C.
5. Gas incubate **incubation** media with 5% CO₂ before storing.
6. Make fresh from stock every 2 - 3 weeks.
7. BSA is cell culture grade fraction V.

Ordering Information:

NOTE: all items used in embyo work are preferably cell culture grade.

CHEMICALS:

ITEM	SUPPLIER	CATA#	INFORMATION
HEPES	Sigma	H9136	100g
Phenol Red	Sigma	P4758	5g
Na Bicarb.	Sigma	S5761	500g
NaCl	Sigma	S5886	500g
KCl	Sigma	P5405	500g
KH ₂ PO ₄	Sigma	P5655	100g
MgSO ₄	Sigma	M2643	500g
Glucose	Sigma	G7021	1Kg
Na pyruvate	Sigma	P2256	
Ca lactate	Sigma	L2000	
DL-lactic acid	Sigma	L1375	
Penn/Strep	Gibco	600-5140AG	
Na Lactate	Sigma		
BSA fraction V	Sigma	A4919	
or BSA frac. V	Sigma	A9647	
dH ₂ O	Sigma	P6757	6x 100ml
or dH ₂ O	Sigma	W3500	
PMS*	Sigma	G4877	Gonadotropin
hCG*	Sigma	CG-2	,orPregnyl
Hyaluronidase	Sigma	H3884	type IV-S:
Mineral oil	Sigma	400-5	heavy white
Flourinert	Sigma	F4758	
Sigmacote	Sigma	SL-2	
Sodium Pentobarbitol			
Propylene glycol			
LMP Agarose			
NACS pre-pacBRL			

DISSECTING TOOLS:

ITEM	SUPPLIER	CATA#	INFORMATION
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Scissors	Thomas	3867-Y15	114mm Miltex
Scissors	Thomas	3867-S20	114mm
Forceps	Thomas	3865-N25	
Forceps	Thomas	386-N30	
Forceps type V	Thomas	3865-J45	
Autoclip applier	Fisher	01-804	
Autoclip remover	Fisher	01-804-15	
Autoclip clips	Fisher	01-804-5	
Serrifine forceps	Thomas	3865-E75	

OTHER SUPPLIES:

7mm hose for micromanipulator collars: **Leitz PO# 0264-000-25010**

50cc glass syringe with ground glass plunger

60cc plastic syring

Tygon tubing 3/32" i.d., 5/32" o.d.

Mouth pipetting tubing (TVD gets them with VWR micropipets)

9" Pastuer pipets or hard glass capillary tubing (1.5mm o.d.)

Microflame bunsen burner

Diamond Pencil

60mm and 35mm tissue culture/petri dishes

1cc tubercular syringes

26g sterile needles

Glass capillaries for injection pipets:

Clark Electro-medical Instuments or **World Preceition Instruments**

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Pangbourne, Reading England

New Haven, CT 06513

RG8-7HU

Cata# TW100F-4

Cata# GC100TF-15

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