
MURINE VENTRICULAR CARDIOMYOCYTE ISOLATION SOLUTIONS COMPOSITION

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IonOptix Protocols

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LIST OF CHEMICALS AND REAGENTS

Chemical	Company	Catalog #
Minimum Essential Medium Eagle (MEM)	Sigma	M0518-10X1L
NaHCO ₃	Sigma	S8875-500G
Pyruvic Acid	Sigma	P-2256
Na-HEPES	Sigma	H7006-500G
HEPES	Sigma	H3375-1KG
Heparin		
Penicillin-Streptomycin (10,000U pen + 10,000mg strep per mL)	Gibco/Invitrogen	15140-122
Albumin from bovine serum (BSA)	Sigma	A6003-100G
Taurine	Sigma	T0625-100G
Liberase TH Research Grade	Roche	54 011 510 001
2,3-Butanedione monoxime	Sigma	
Creatine	Sigma	
Human Insulin	Internal Hospital Pharmacy	



SOLUTIONS TO PREPARE PRIOR TO DAY OF EXPERIMENTS

Liberase TH Research Grade aliquots

- Dissolve 50 mg of Liberase TH in 10 mL dH₂O
- Sit on ice to dissolve for 30 minutes
- Separate into 1.125 mL aliquots (2.25 mL of 5 mg/mL = 11.25 mg)
2.25 mL will be added to 500 mL enzyme perfusion solution
- Freeze at -80 until day of experiment

Zero Ca²⁺ PSS Base Solution

<u>Chemical</u>	<u>FW or fluid conc</u>	<u>Amount /2L</u>	<u>final [mM]</u>
NaCl	58.44	16.71 g	143
KCl	74.55	0.7455 g	5
d-Glucose	180	3.6 g	10
HEPES	238.3	4.766 g	10
MgCl ₂	1 M sln	2 mL	1

fill to ~2L
stir, pH with NaOH to 7.35, fill to 2L
Store at 4 degrees, make fresh weekly

MEM Base Solutions

<u>Chemical</u>	<u>FW or fluid conc</u>	<u>Amount/L</u>	<u>final [mM]</u>
MEM	-	11.19 g	1X
PenStrep sln	10,000 U/mL	5 mL	50,000 U/L
NaHCO ₃	84.01	0.84 g	10
Na-Pyruvate	110.04	0.22 g	2
Na-HEPES	260.29	2.6 g	10
HEPES	238.3	2.38 g	10

fill to ~1L
stir, pH to 7.35, fill to 1L
Filter sterilize with 0.22 µm SteriCups
Store at 4 degrees, keep sterile, make fresh weekly



DAY-OF SOLUTIONS

MOUSE VENTRICULAR CARDIOMYOCYTE ISOLATION: LANGENDORFF PROTOCOL

1

Solution 1: Initial Wash

To 100 mL of **0 Ca²⁺ Base** solution add:

200 µl of heparin (1000 U/ml).

Final concentration of solution:

NaCl	143 mM
KCl	5 mM
d-Glucose	5 mM
Hepes	5 mM
MgCl ₂	1 mM
Heparin	2 U/mL

2

Solution 2: Enzyme Perfusion Solution

To **40 mL MEM Base solution** add:

20 mg of BDM (2,3-Butanedione monoxime)

7 µl of 0.1 M CaCl₂ solution

Heat to 37 degrees C

Just prior to use add 350 µL of Blendzyme TH solution.

Final concentration of solution:

MEM	1X
NaHCO ₃	7 mM
Na-Pyruvate	2 mM
Na-HEPES	7 mM
HEPES	7 mM
CaCl ₂	20 µM
Taurine	5 mM
BDM	5 mM
PenStrep	50,000 U/L
Blendzyme TH	0.045 mg/mL

3

Solution 3: BSA Solution

To **30 mL MEM Base solution**
add:

300 mg BSA – allow to dissolve

12 µl of Human Insulin

18.7mg of Taurine

Final concentration of solution:

MEM	1X
NaHCO ₃	7 mM
Na-Pyruvate	2 mM
Na-HEPES	7 mM
HEPES	7 mM
Taurine	10 mM
PenStrep	50,000 U/L

4

Room Temp 0-Ca²⁺
PSS Base

5a

50/50 Adaptor/BSA

To a 15mL falcon tube, mix 2 mL of Solution 4 and 2 mL of Solution 3.

Then add:
3.2 µl of 1M CaCl₂

Final solution concentration
should be 800 µM.

5b

Adaptor with CaCl₂

To a 15mL falcon tube, add
10mL of Solution 4.

Then add:
4.5 µl of 1M CaCl₂

Final solution concentration
should be 450 µM.

DAY-OF SOLUTIONS

RAT VENTRICULAR CARDIOMYOCYTE ISOLATION: LANGENDORFF PROTOCOL

1

Solution 1: Initial Wash

Add 600 μ l of heparin (10,000 U/10ml) to 300 mL **0 Ca²⁺ Base**

Final concentration of solution:

NaCl	143 mM
KCl	5 mM
d-Glucose	10 mM
Hepes	10 mM
MgCl ₂	1 mM
Heparin	2 U/mL

2

Solution 2: Enzyme Perfusion Solution (MEM #1 with Blendzyme and Taurine)

To **100 mL MEM Base solution** add:

100 mg Taurine (MW 125.1)
10 μ l of 0.1 M CaCl₂ solution
200 μ l of heparin (10,000U/10ml)
Heat to 37 degrees, just prior to use add 875 μ L of Blendzyme TH solution.

Final concentration of solution:

MEM	1X
NaHCO ₃	10 mM
Na-Pyruvate	2 mM
Na-HEPES	10 mM
HEPES	10 mM
CaCl ₂	10 μ M
Taurine	8 mM
PenStrep	50,000 U/L
Blendzyme TH	0.045 mg/mL

3

Solution 3: BSA Solution (MEM #2 with BSA)

To **50 mL MEM Base solution** add:

0.500 g BSA

Final concentration of solution:

MEM	1X
NaHCO ₃	10 mM
Na-Pyruvate	2 mM
Na-HEPES	10 mM
HEPES	10 mM
CaCl ₂	50 μ M
PenStrep	50,000 U/L
BSA	10 mg/mL

4

Solution 4: Final Wash Solution (MEM #2 with Taurine)

To **50 mL MEM Base solution** add:

62.5 mg Taurine (MW 125.1)
25 μ l of 0.1 M CaCl₂ solution.

Final concentration of solution:

MEM	1X
NaHCO ₃	10 mM
Na-Pyruvate	2 mM
Na-HEPES	10 mM
HEPES	10 mM
CaCl ₂	50 μ M
Taurine	10 mM
PenStrep	50,000 U/L

5a

50/50 Adaptor/BSA

To a 50mL falcon tube, mix 25mL of Solution 4 and 25mL of Solution 3.

Then add:
40 μ l of 1M CaCl₂

Final solution concentration should be 800 mM of 1M CaCl₂.

5b

Adaptor with CaCl₂

To a 50mL falcon tube, add 50mL of Solution 4.

Then add:
22.5 μ l of 1M CaCl₂

Final solution concentration should be 450 mM of 1M CaCl₂.