Porcine Cardiomyocyte Isolation Solutions Composition

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IonOptix Protocols

09/30/2019

List of Chemicals and Reagents

Chemical	Company	Catalog #
Minimum Essential Medium Eagle (MEM)	Sigma	M0518-10X1L
NaHCO3	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

SOLUTIONS TO PREPARE PRIOR TO DAY OF EXPERIMENTS

Liberase TH Research Grade aliquots

-Dissolve 50 mg of Liberase TH in 10 mL dH_2O
-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 Ca^{2+} PSS Base Solution

Chemical	FW or fluid conc	Amount /2L	final [mM]
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

Chemical	FW or fluid conc	Amount/L	final [mM]
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

Porcine cardiomyocyte isolation: Left-Ventricular wedge Preparation

1

Solution 1: Initial Wash

To **500ml of 0 Ca^{2+} Base Solution**, add:

1 ml 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

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Solution 2: Enzyme Perfusion Solution

To **500 mL MEM Base solution** add:

500 mg Taurine (MW 125.1)
100 µl of 0.1 M $CaCl_2$ solution
1 ml of heparin (10,000U/10ml)
Heat to 37 degrees, just prior to use add 2.25 mL 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	10 mM
PenStrep	50,000 U/L
Blendzyme TH	0.045 mg/mL

Sol 1:15 min
Sol 2: 28 min

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Solution 3: BSA Solution

To **200 mL MEM Base solution** add:

2 g BSA
200 µl of 0.1 M $CaCl_2$ 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 µM
PenStrep	50,000 U/L
BSA	10 mg/mL

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Solution 4: Final Wash Solution

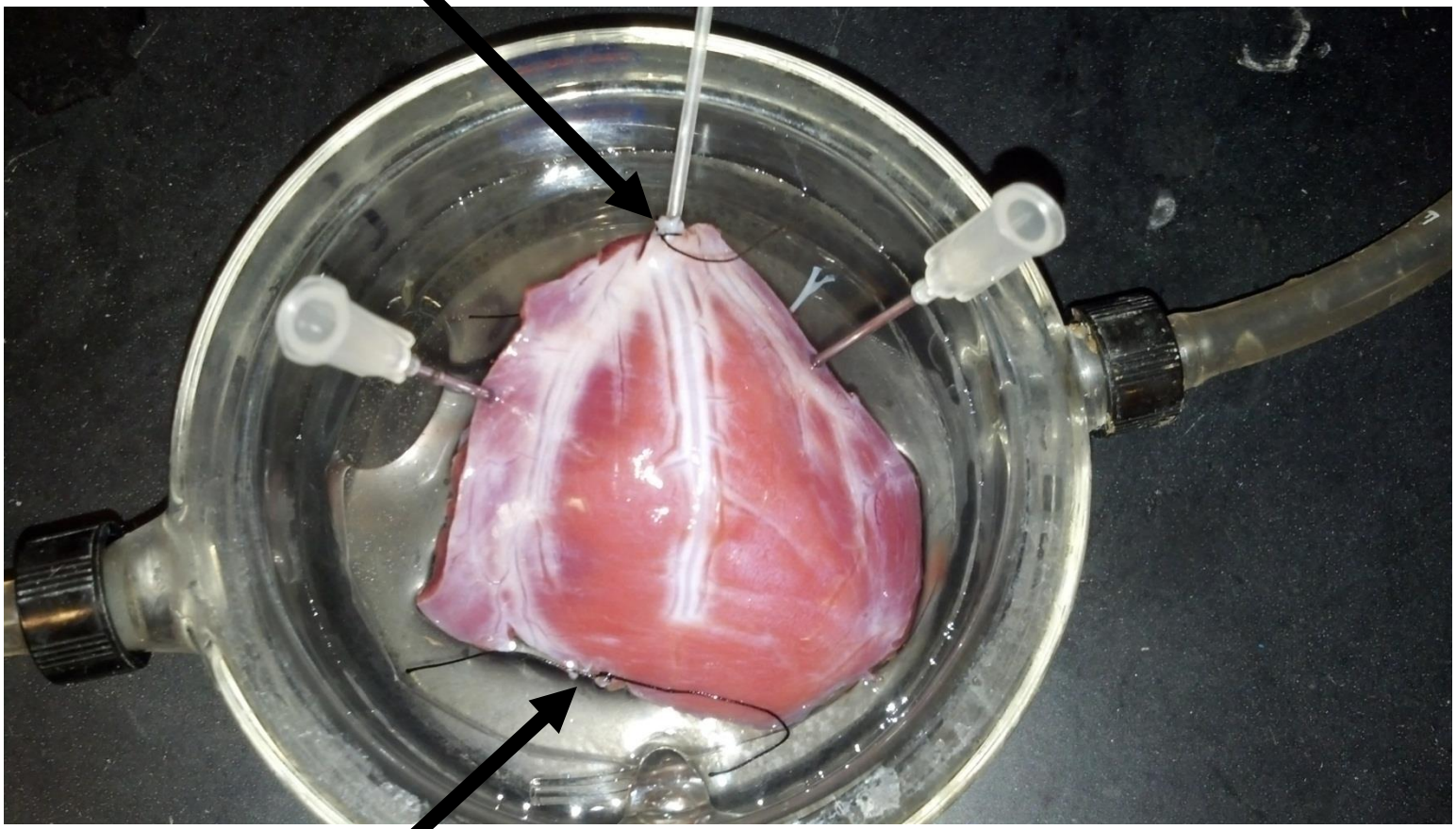
To **100 mL MEM Base solution** add:

125 mg Taurine (MW 125.1)
48 µl of 0.1 M $CaCl_2$ 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 µM
Taurine	10 mM
PenStrep	50,000 U/L

LAD Cannulation

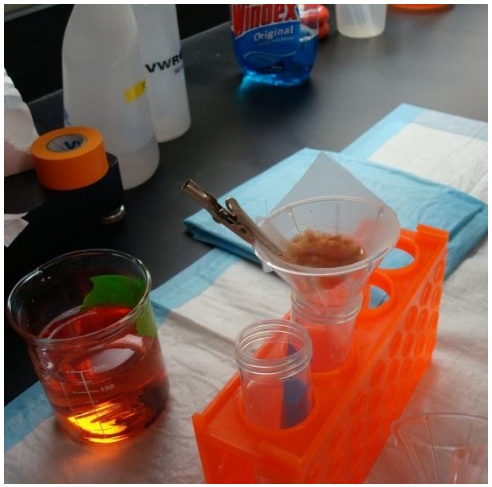


Coronary ligations

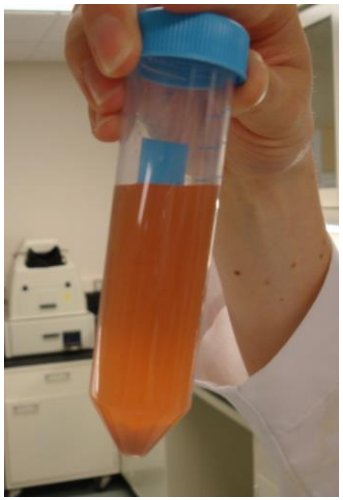
Myocyte Isolation

VENTRICULAR MYOCYTES (MID-MYOCARDIUM):

- At ~30 minutes post digestion, cut through center of LV wedge. If color (orange) and texture (soft but not slimy) of a region is appropriate, cut out 1 inch square section and place in Solution 3. Remove epi and endocardium, and place mid-myocardial region into fresh Solution 3 in a Medium weight boat. Cut into smaller segments with fine scissors. (Note: Sharpness of scissors matters, otherwise you crush cells and live/dead ratio will drop. Also, don't dice tissue, just cut into smaller segments so cells can slough off). Gently shake tissue with forceps for 3 minutes and cells will come off.
- Filter cells through 200 μm Nylon Filter Mesh into 50 mL Falcon Tube
- Place Falcon Tube (lid on but open to atmosphere) at ~30 degree angle for 5 minutes, and cells will settle on the bottom/side of tube.
- Turn tube upright and gently twist tube by hand to release cells from the side, and they will gradually form a soft pellet at the bottom of the tube.
- Immediately Resuspend pellet in 35 mL ***Solution 3***, and check density and quality of cells.
- Allow cells to settle (30 degrees) and pellet (twist tube) again.
- Resuspend this pellet in 10-40 mL of ***Solution 4***, depending on how dense you want the cells.



Filter through 0.2 mm Nylon mesh into 50 mL Falcon Tube



Example ventricular myocyte pellet