

**VERSION 3** 

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# Human Islet Isolation Media Preparation V.3

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#### **ABSTRACT**

This protocol describes the preparation of various media formulations required for the isolation and culture of human islets, as performed by the Alberta Diabetes Institute IsletCore. <a href="http://www.bcell.org/adi-isletcore.html">http://www.bcell.org/adi-isletcore.html</a>

#### **MATERIALS**

- X Hanks balanced salts powder Corning Catalog #55-022-PB
- Minimum Essential Medium Eagle modifided (EMEM) powder Corning Catalog #90-009PB
- Medium 199 powder Corning Catalog #90-050-PC
- Sodium Bicarbonate Fisher Scientific Catalog #S233

- **⋈** HEPES Fisher Scientific Catalog #BP310
- Penicillin/streptomycin Lonza Catalog #09-757F
- Albumin Bovine Serum (30%) equitech bio, inc. Catalog #160928-0262
- **☒** Betadine Solution (10%) **Stevens company Catalog #036-08617**

Lympolyte 1.1, Cell Separation Media 1.10 g/ml - (Cedarlane Catalogue #CL6255)

- Belzer UW Cold Storage Solution Bridge to Life Catalog #092614
- X CMRL 1066 medium Corning Catalog #15-110-CV
- Signature (100x) Gibco Thermo Fischer Catalog #35050-061

- Stericup Sterile Vacuum Filtration System. **Fisher Scientific Catalog** #**\$2GPU05RE**
- **∅** 0.45um Syringe Filter **Fisher Scientific Catalog #09-740-116**

## HBSS (perfusion, rinse, priming solution), M199 (wash solu...

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A	В	С	D	E
Hank's Balanced Salt Solution - HBSS (perfusion, rinse, priming solutions) (10L)				
Reagent	Concentration	Weight/Volum e	Supplier	Catalogue #
HBSS Powder	9.51 g/L	1 bottle	Mediatech/Corni ng	55-022-PB

A	В	С	D	E
CaCl2 (anhydrous)	3.60 mM	4.0g	MP Biochemicals LLC	15350290
MgSO4 (anhydrous)	0.81 mM	0.98g	VWR Life Science	97061-438
NaHCO3	4.2 mM	3.5g	Fisher Scientific	S233-500
HEPES	10mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomyci n	100 U/ml penicillin 100 µg/ml streptomycin	50ml	Lonza	09-757F

A	В	С	D	E
Medium 199 - M199 (Wash media) (10L)				
Reagent	Concentration	Weight/volum e	Supplier	Catalogue #
M199 powder	9.41g/L	1 bottle	Mediatech/Corni ng	90050PB
NaHCO3	26 mM	22.0g	Fisher Scientific	S233-500
HEPES	10 mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomyc in	100 U/ml penicillin 100 µg/ml streptomycin	50ml	Lonza	09-757F

A	В	С	D	E
Eagle Modified Minimal Essential Medium - EMEM (Dilution Media) (10L)				
Reagent	Concentration	Weight/volum e	Supplier	Catalogue #
EMEM powder	9.23g/L	1 bottle	Mediatech/Cornin g	90009BP
NaHCO3	26 mM	22.0g	Fisher Scientific	S233-500
HEPES	10 mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomycin	100 U/ml penicillin 100 µg/ml streptomycin	50ml	Lonza	09-757F

# Prepare and pH the HBSS, M199 and EMEM solutions

- 2 1. Prepare the HBSS, M199, and EMEM solutions outlined in step 1 of the Stock Media Preparation table using the following directions:
  - 2. Dispense  $\underline{L}$  9  $\underline{L}$  of Milli-Q (18m $\Omega$ ) water in to the carboy
  - 3. Store overnight at 4 °C to allow to come to temperature.
  - 4. Using the stirrer add the media powder to the water and allow to go into solution.
  - 5. Add the powdered supplements and Penicillin/Streptomycin to the appropriate media based on the above tables and allow to stir into solution.
  - 6. Stir the solution for 00:30:00
  - 7. Store the prepared solution overnight at 4 °C to allow all powders to go into solution
  - 8. Stir the solution for 00:30:00
  - 9. Calibrate the pH meter using the pH control buffers
  - 10. Adjust the pH level to PH 7.4 using the NaOH and/or HCl solutions.
  - 11. Bring to volume with the appropriate amount of Milli-Q water.

### Filter sterilize the HBSS, M199 and EMEM solutions

- 3 1. Sterile filter using the peristaltic pump, the tubing (silicon 25 gauge) and the capsule filter (Nylon Capsule filter 0.22µm membrane) into the appropriate sterilized media bottle.
  - 2. Store the filtered media at 4 °C until needed for supplementation prior to isolation.

## Prepare, pH and filter sterilize the 1M Nicotinamide solution

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A	В	С	D	E
Nicotinamide Solution - 1M (1L)				
Reagent	Concentration	Weight/volume	Supplier	Catalogue #
Nicotinamide powder	1 M	122.12	Sigma Aldrich Canada Co	N0636
HEPES	10 mM	2.383g	Fisher Scientific	BP310-1

- 5 1. Dispense 4 0.9 L of Milli-Q water in to the carboy.
  - 2. Store overnight at 4 °C to allow to come to room temperature.
  - 3. Using the stirrer add the reagent powder into the water based on the table (Step 4) and allow to stir into solution.
  - 4. Stir the solution for 00:30:00
  - 5. Calibrate the pH meter using the pH control buffers
  - 6. Adjust the pH level to 6. Adjust the pH level to 6. Adjust the pH level to 6. The first of the base of the pH level to 6. Adjust the pH level to 6. Adjust the pH level to 6. The first of the pH level to 6. The pH level
  - 7. Bring to volume (1L) with the appropriate amount of Milli-Q water.
  - 8. Filter sterilize using a bottle top filter (0.22µM) into a sterile 1L bottle.

# Preparation and use of Dithizone stain in Islet Preparations.

### 6 Preparation of dithizone

- 1. Weigh out  $\mathbb{Z}_{0.2 \text{ g}}$  of dithizone powder into a 50ml conical tube.
- 2. Add A 6 mL of DMSO and mix until the powder is in solution.
- 3. Bring the resulting dithizone solution to 40ml total volume with HBSS and mix.
- 4. Transfer the dithizone solution to a 60cc syringe with a 0.45µm nylon filter.

#### Use

- 1. For every ml of islet suspension add an equal amount of the prepared dithizone solution must be added to the sample.
- 2. For visualization of staining add another L 2 mL of HBSS to dilute the stain and reduce the background colour.

# Preparation of working solutions to be done the day of isola.

7 To prepare the working solution supplement each of the listed stock medias with the indicated supplements.

A	В	С	D	E	F	G	Н
	Total volume per bottle	BSA 30%	HBSS	EMEM	M199	Nicotinamide (1M)	Betadine
	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)

A	В	С	D	E	F	G	Н
SOLUTION							
M199 (aliquot into 12 x 100ml)	1172	160 (4.0%)			1000	12	
Dilution - make 3 bottles	2020			2000		20	
Priming solution	1000		1000				
Wash 1 - split into 2x 550ml	1105	95 (2.5%)			1000	10	
Wash 2 - make 5 bottles	1055	45 (1.125% )			1000	10	
Cannulation	500		500				
Perfusion Solution	350		350				
Decon 1	300		250				50
Decon 2	250		250				
Decon 3	250		250				

### Collection tubes for collection of tissue during the purification step.

A	В	С	D	E	F	G
Gradient Collection tubes						
tube #	1	2	3	4	5	6
ml of wash 2 solution	100	150	200	200	225	225
tube #	7	8	9	10	11	12
ml of wash 2 solution	225	225	225	225	225	150

# Preparation of gradient solutions for purification

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A	В	С	D	E
Density Gradients				
	lympholyte 1.100g/ml	UW sol'n. 1.042 g/ml	Volume (ml)	Final Density g/ml
High density	77	64	141	1.074
Low density	52	88	140	1.064

### **Human Islet Culture media**

### 9 Preparation of the human islet culture media

Prepare the culture media by supplementing CMRL by the following table. Following these additions to the culture media, filter sterilize the resulting solution through a 0.22  $\mu$ m Stericup Sterile Vacuum Filtration System.

A	В	С	D	E	F
	CMRL 1066	Bovine serum albumin 30%	Insulin- Transferrin- Selenium (100x)	Gibco® GlutaMAX Supplement	Penicillin- Streptomycin Mixture
	(ml)	(ml)	(ml)	(ml)	(ml)
Culture media	500	8.5	5	10	2.5

CMRL 1066 - Corning Catalogue # 15110CV

Bovine Serum Albumin 30% v/v - Equitech Bio Inc. Catalogue # BAL62

Insulin-Transferrin-Selenium (100x) - Corning Catalogue # 25800CR

Gibco® GlutaMAX™ Supplement (100x) - ThermoFisher catalogue # 35050061

Penicillin-Streptomycin Mixture - Lonza Catalogue # <u>09-757F</u>