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Version 1

Human Islet Isolation Media Preparation V.1

 Endocrinology

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DOI

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Human Cell Atlas Metho...

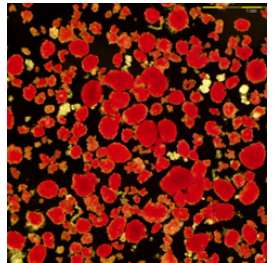
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Protocol status: Working

We use this protocol and it's working

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Keywords: human islet isolation media preparation, preparation



Materials

MATERIALS

- ✕ Hanks balanced salts powder **Corning Catalog #55-022-PB**
- ✕ Minimum Essential Medium Eagle modified (EMEM) powder **Corning Catalog #90-009PB**
- ✕ Medium 199 powder **Corning Catalog #90-050-PC**
- ✕ Sodium Bicarbonate **Fisher Scientific Catalog #S233**
- ✕ Calcium Chloride **MP Biomedicals Catalog #153502**
- ✕ Magnesium Sulfate **Amresco Catalog #0338**
- ✕ 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**
- ✕ Nicotinamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #N0636**
- ✕ Biocoll 1.100 **Biochrom AG Catalog #L 6155**
- ✕ Belzer UW Cold Storage Solution **Bridge to Life Catalog #092614**
- ✕ CMRL 1066 medium **Corning Catalog #15-110-CV**
- ✕ Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**
- ✕ Insulin Transferrin Selenium (20x) **Corning Catalog #25-800-CR**
- ✕ Collagenase **vitacyte Catalog #011-1060**
- ✕ Non-specific protease powder **Roche Catalog #05339880001**
- ✕ Dimethyl Sulfoxide **Fisher Scientific Catalog #D128**
- ✕ Nylon capsule filter 0.22uM **maine manufacturing Catalog #1213757**
- ✕ Dithizone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #43820**
- ✕ DNase DNASE I GRADE II **Roche Catalog #10104159001**
- ✕ Stericup Sterile Vacuum Filtration System. **Fisher Scientific Catalog #S2GPU05RE**
- ✕ 0.45um Syringe Filter **Fisher Scientific Catalog #09-740-116**

HBSS (perfusion, rinse, priming solution), M199 (wash solution) and EMEM (dilution solution)

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HBSS (perfusion, rinse, priming solutions) (10L)				
Reagent	Concentration	Weight/Volume	Supplier	Supplier Catalogue #
HBSS Powder	9.51 g/L	1 bottle of HBSS powder		55-022-PB
CaCl ₂ (anhydrous)	3.60 mM or 0.4 g/L	4.0g	MP Biochemicals LLC	15350290
MgSO ₄ (anhydrous)	0.81 mM or 0.0977 g/L	0.98 g	VWR Life Science	97061-438
NaHCO ₃	4.2 mM	3.5g	Fisher Scientific	S233-500
HEPES	10mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomycin 20,000 units potassium penicillin and 20,000 µg streptomycin sulfate per mL in 0.85% saline	100 U/ml penicillin and 100 µg/ml streptomycin	50ml	Lonza	09-757F



<u>M199 (wash solutions 1 and 2) (10L)</u>			Supplier	Supplier Catalogue #
<u>Reagent</u>	<u>Concentration</u>	<u>Weight/volume</u>		
M199 powder	9.41g/L	1 bottles (1×10L/bottle)	Mediatech/Corning	90050PB
NaHCO₃	26 mM	22.0g	Fisher Scientific	S233-500
HEPES	10 mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomycin 20,000 units potassium penicillin and 20,000 µg streptomycin sulfate per mL in 0.85% saline	100 U/ml penicillin and 100 µg/ml streptomycin	50ml	Lonza	09-757F

<u>EMEM (dilution solution) (10L)</u>			Supplier	Supplier Catalogue #
<u>Reagent</u>	<u>Concentration</u>	<u>Weight/volume</u>		
EMEM powder	9.23g/L	1 bottles (1×10L/bottle)	Mediatech/Corning	90009BP



NaHCO₃	26 mM	22.0g	Fisher Scientific	S233-500
HEPES	10 mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomycin 20,000 units potassium penicillin and 20,000 µg streptomycin sulfate per mL in 0.85% saline	100 U/ml penicillin and 100 µg/ml streptomycin	50ml	Lonza	09-757 F

Prepare and pH the HBSS, M199 and EMEM solutions

- 2
 1. Prepare the HBSS, M199, and EMEM solutions outlined in step 1 – Stock Media Preparation table using the following directions:
 2. Dispense 9L of Milli-Q (18mΩ) 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 table (step 1) and allow to stir into solution.
 6. Stir the solution for 30 min
 7. Store the prepared solution overnight at 4°C to allow all powders to go into solution
 8. Stir the solution for 30 min
 9. Calibrate the pH meter using the buffer controls
 10. Adjust the pH level to 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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<u>Nicotinamide (1L)</u>			Sup plier	Sup plier Cata logu e #
<u>Reagent</u>	<u>Concentration</u>	<u>Weight/volume</u>		
Nicotinamide powder	1 M	122.12	Sig ma Aldri ch Can ada Co	N06 36
HEPES	10 mM	2.383g	Fish er Scie ntific	BP31 0-1

- 5
1. Dispense 0.9L 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 30 min
 5. Calibrate the pH meter using the buffer controls
 6. Adjust the pH level to 7.4 using the NaOH and/or HCl solutions
 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 0.2g of dithizone powder into a 50ml conical tube.
2. Add 6mls 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 2mls of HBSS to dilute the stain and reduce the background colour.
3. Alternately, 100µl of islet suspension, 100µl dithizone and 200µl HBSS.

Preparation of working solutions to be done the day of isolation.

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

Media	Total per bottle	BSA 25%	HBS S	EME M	M199	HBSS Perifusion	HBSS (priming)	Nicotina mide
	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)
M199 (aliquot x12)	1172	160 (4.0 %)	-----	-----	1000	-----	-----	12
Dilution #1, #2, #3	2020 (x3)	-----	-----	2000 (x3)	-----	-----	-----	20
Priming solution	1000	-----	-----	-----	-----	-----	1000	-----
Wash 1 - split into 2x 550ml	1105 (x1)	95 (2.5 %)	-----	-----	1000	-----	-----	10
Wash 2	1055 (x5)	45 (1.125 %)	-----	-----	1000 (x5)	-----	-----	10
Cannulation	500	-----	500	-----	-----	-----	-----	-----
Perfusion Solution	350	-----	-----	-----	-----	350	-----	-----
Decon 1	250	-----	200	Beta dine 50mL	-----	-----	-----	-----
Decon 2	250	-----	250	-----	-----	-----	-----	-----
Decon 3	250	-----	250	-----	-----	-----	-----	-----

Collection tubes for collection of tissue during the purification step.

Gradient Collection tubes						
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tube #	1	2	3	4	5	6
mL of wash solution	100	150	200	200	225	225
tube #	7	8	9	10	11	12
mL of wash solution	225	225	225	225	225	150

Prepare the culture media by supplementing CMRL by the following table. Following these additions to the culture media, filter sterilize using the Stericup Sterile Vacuum Filtration System.

Media	Total per bottle	CMRL	BSA 30%	ITS (100x)	Glutam ax	Pen/strep
Culture Media	521	500	8.5 (0.5%)	5	5	2.5