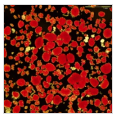

Human Islet Isolation Media Preparation

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Hanks balanced salts powder	55-022-PB	Corning
Minimum Essential Medium Eagle modifided (EMEM) powder	90-009PB	Corning
Medium 199 powder	90-050-PC	Corning
Sodium Bicarbonate	S233	Fisher Scientific
Calcium Chloride	153502	MP Biomedicals
Magnesium Sulfate	0338	Amresco
HEPES	BP310	Fisher Scientific
Penicillin/streptomycin	09-757F	Lonza
Albumin Bovine Serum (30%)	160928-0262	equitech bio, inc.
Betadine Solution (10%)	036-08617	Stevens company
Nicotinamide	N0636	Sigma Aldrich
Biocoll 1.100	L 6155	Biochrom AG
Belzer UW Cold Storage Solution	092614	Bridge to Life
CMRL 1066 medium	15-110-CV	Corning
Glutamax (100x)	35050-061	Gibco - Thermo Fischer
Insulin Transferrin Selenium (20x)	25-800-CR	Corning
Collagenase	011-1060	vitacyte
Non-specific protease powder	05339880001	Roche
Dimethyl Sulfoxide	D128	Fisher Scientific
Nylon capsule filter 0.22uM	1213757	maine manufacturing
Dithizone	43820	Sigma-aldrich
DNase DNASE I GRADE II	10104159001	Roche
Stericup Sterile Vacuum Filtration System.	S2GPU05RE	Fisher Scientific
0.45um Syringe Filter	09-740-116	Fisher Scientific

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

1

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
CaCl2 (anhydrous)	3.60 mM or 0.4 g/L	4.0g	MP Biochemicals LLC	15350290
MgSO4 (anhydrous)	0.81 mM or 0.0977 g/L	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/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

M199 (wash solutions 1 and 2) (10L)			Supplier	Supplier Catalogue #
Reagent	**Concentration**	**Weight/volume**		
M199 powder	**9.41g/L**	**1 bottles (1x10L/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

EMEM (dilution solution) (10L)			Supplier	Supplier Catalogue #
Reagent	**Concentration**	**Weight/volume**		
EMEM powder	**9.23g/L**	**1 bottles (1x10L/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	09-757F

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

4

Nicotinamde (1L)			Supplier	Supplier Catalogue #
Reagent	**Concentration**	**Weight/volume**		
Nicotinamide powder	**1 M**	**122.12**	Sigma Aldrich Canada Co	N0636
HEPES	**10 mM**	**2.383g**	Fisher Scientific	BP310-1

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%	HBSS	EMEM	M199	HBSS Perifusion	HBSS (priming)	Nicotinamide
	(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	Betadine 50mL	-----	-----	-----	-----
Decon 2	250	-----	250	-----	-----	-----	-----	-----
Decon 3	250	-----	250	-----	-----	-----	-----	-----

Collection tubes for collection of tissue during the purification step.

Gradient Collection tubes						
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 sterilze using the Stericup Sterile Vacuum Filtration System.

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