Version 2

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Human Islet Isolation Media Preparation V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bt6enrbe

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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. http://www.bcell.org/adi-isletcore.html

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WHAT'S NEW

Minor edits for clarity.

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49062

MATERIALS TEXT

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 **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 Fischer Catalog #35050-061**

Insulin Transferrin Selenium (20x) **Corning Catalog #25-800-CR**

Dimethyl Sulfoxide **Fisher Scientific Catalog #D128**

Nylon capsule filter 0.22uM **maine manufacturing Catalog #1213757**

Dithizone **Sigma-aldrich Catalog #43820**

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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A	B	C	D	E
Hank's Balanced Salt Solution - HBSS (perfusion, rinse, priming solutions) (10L)				
Reagent	**Concentration**	**Weight/Volume**	**Supplier**	**Catalogue #**
HBSS Powder	9.51 g/L	1 bottle	Mediatech/Corning	55-022-PB
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/Streptomycin	100 U/ml penicillin 100 µg/ml streptomycin	50ml	Lonza	09-757F

A	B	C	D	E
Medium 199 - M199 (Wash media) (10L)				
Reagent	**Concentration**	**Weight/volume**	**Supplier**	**Catalogue #**
M199 powder	9.41g/L	1 bottle	Mediatech/Corning	90050PB
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

A	B	C	D	E
Eagle Modified Minimal Essential Medium - EMEM (Dilution Media) (10L)				
Reagent	**Concentration**	**Weight/volume**	**Supplier**	**Catalogue #**
EMEM powder	9.23g/L	1 bottle	Mediatech/Corning	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 **9 L** 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 above tables

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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

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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	B	C	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

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1. Dispense **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 **pH 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 **0.2 g** of dithizone powder into a 50ml conical tube.
2. Add **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.

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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 **2 mL** of HBSS to dilute the stain and reduce the background colour.
3. Alternately, **100 µl** of islet suspension, **100 µl** dithizone and **200 µl** 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.

A	B	C	D	E	F	G	H
	Total volume per bottle	**BSA 30%**	**HBSS**	**EMEM**	**M199**	**Nicotinamide (1M)**	**Betadine**
	(ml)	**(ml)**	**(ml)**	**(ml)**	**(ml)**	**(ml)**	**(ml)**
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	B	C	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

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Human Islet Culture media

8 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 µm Stericup Sterile Vacuum Filtration System.

A	B	C	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 # 09-757F

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