



Apr 29, 2021

Human Islet Isolation Enzyme Preparation Version 1.0

In 2 collections

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1 Works for me dx.doi.org/10.17504/protocols.io.budcns2w

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ABSTRACT

This protocol describes the preparation of enzymes used in the isolation of human research islets by the Alberta Diabetes Institute IsletCore (www.bcell.org/adi-isletcore). It is to be used in concert with the other islet isolation protocols published by IsletCore.

The ADI IsletCore program is described in full here:

Lyon J, Manning Fox JE, Spigelman AF, Kim R, Smith N, O'Gorman D, Kin T, Shapiro AMJ, Rajotte RV, MacDonald PE (2016) Research-focused isolation of human islets from donors with and without diabetes at the Alberta Diabetes Institute IsletCore. [Endocrinology, 157\(2\): 560-569.](#)

DOI

dx.doi.org/10.17504/protocols.io.budcns2w

PROTOCOL CITATION

James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald 2021. Human Islet Isolation Enzyme Preparation Version 1.0. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.budcns2w>

COLLECTIONS ⓘ

 **ADI IsletCore Protocols for the Isolation, Assessment and Cryopreservation of Human Pancreatic Islets of Langerhans for Research Purposes** **ADI IsletCore Protocols for the Isolation, Assessment and Cryopreservation of Human Pancreatic Islets of Langerhans for Research Purposes**

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CREATED

Apr 20, 2021

LAST MODIFIED

Apr 29, 2021

PROTOCOL INTEGER ID

49284

PARENT PROTOCOLS

Part of collection

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MATERIALS TEXT

☒ Hanks balanced salts

powder **Corning Catalog #55-022-PB**

☒ 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**

☒ Collagenase

vitacyte Catalog #011-1060

☒ Non-Specific Protease - AOF BP

Protease **vitacyte Catalog # 003-2000**

☒ DNase DNASE I GRADE II

Roche Catalog #10104159001

☒ Nylon capsule filter 0.22uM **maine**

manufacturing Catalog #1213757

Preparation of HBSS Perfusion Solution - 10L

1

A	B	C	D	E
Hank's Balanced Salt Solution - HBSS (perfusion, rinse, priming solutions) (10L)				
Reagent	Final Concentration	Weight/Volume	Supplier	Catalogue Number
HBSS Powder	9.51 g/L	1 bottle	Mediatech/Corning	55-022-PB
CaCl ₂ (anhydrous)	3.60 mM	4.0g	MP Biochemicals LLC	15350290
MgSO ₄ (anhydrous)	0.81 mM	0.98g	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	100 U/ml penicillin 100 µg/ml streptomycin	50ml	Lonza	09-757F

1. Dispense **9 L** of Milli-Q (18mΩ) water into the carboy
2. Store overnight at **4 °C** to allow to come to temperature.
3. Using the stirrer add the media powder to the water and allow it to go into solution.
4. Add the powdered supplements and penicillin/streptomycin to the appropriate media based on the above table and stir to allow it to go into solution.
5. Stir the solution for **00:30:00**
6. Store the prepared solution overnight at **4 °C** to allow all powders to go into solution
7. Stir the solution for **00:30:00**
8. Calibrate the pH meter using the pH control buffers
9. Adjust the pH level to **pH7.4** using the NaOH and/or HCl solutions.
10. Bring to volume with the appropriate amount of Milli-Q water.
11. 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.
12. Store the filtered media at **4 °C** until needed for supplementation prior to isolation.

Reconstitution of Collagenase

- 2
 1. Obtain one vial collagenase powder from **-20 °C** storage and place it on ice.
 2. Under sterile conditions add **30 mL** of perfusion solution to the vial of collagenase powder.
 3. Do not mix but allow the lyophilized cake of collagenase to settle into the solution for **00:30:00**, gently swirling the bottle every 10 minutes to be sure to allow any residual collagenase that might be adhered to the glass to go into solution.
 4. After 30 min sterilely transfer the resulting collagenase concentrate to **200 mL** of perfusion solution in a sterile 500 ml media bottle (on-ice)
 5. Rinse the original collagenase bottle with **20 mL** of perfusion solution and transfer the rinse to the media bottle.
 6. Repeat step 5 until you are satisfied that all of the collagenase solution is now in the media bottle - 3x is sufficient
 7. Bring the total volume of the now working collagenase solution to approximately **350 mL** using perfusion solution.

8. Continue to store on ice until Step 7 of the [Isolation of Human Pancreatic Islets of Langerhans for Research](#) protocol.

Reconstitution of Neutral Protease

- 3
 1. Obtain a vial of non-specific protease powder from -20°C storage and place it on ice.
 2. Under sterile conditions remove the rubber stopper from the vial add **10 mL** of perfusion solution.
 3. Reseal the vial with the rubber stopper and invert slowly ~5 times. Do not shake.
 4. Continue to store the vial on ice until Step 7 of the [Isolation of Human Pancreatic Islets of Langerhans for Research](#) protocol.
 5. Once the weight of the pancreas has been determined (see Step 7 of the [Isolation of Human Pancreatic Islets of Langerhans for Research](#)), remove a volume of non-specific protease solution that will equal to 25,000 U of enzyme per gram of pancreas weight.

The exact number of enzymatic units in each vial is lot dependant but the amount the number of units is stated by the manufacturer on the provided certificate of analysis. This will be stated as 25,000,000 U/vial +/- 200,000 U. For example a 100 g pancreas will require the full 10 ml of the non-specific protease solution to achieve 25,000 U/g of pancreas. A 50 g pancreas will require 5 ml of the non-specific protease solution to achieve 25,000 U/g of pancreas. The amount of non-specific protease should be adjusted to 12,500 U/g of pancreas when the cold ischemia time is greater than 10 hrs.

Preparation and use of the Collagenase / Non-Specific protease Solution

- 4
 1. Once you have determined the volume of the non-specific protease solution required, transfer this volume to the 350 ml of the collagenase solution in step 2.
 2. Store the resulting solution on ice until Step 7(5) of the [Isolation of Human Pancreatic Islets of Langerhans for Research](#) protocol.

Preparation and use of DNase Solution

- 5
 1. Obtain one vial of DNase I Grade II from bovine pancreas from 4°C storage and place it on ice.
 2. Under sterile conditions remove the rubber stopper from the vial add **4 mL** of perfusion solution.
 3. Reseal the vial with the rubber stopper and invert slowly ~5 times. Do not shake.
 4. Continue to store the vial on ice until Step 8(6) of the [Isolation of Human Pancreatic Islets of Langerhans for Research](#) protocol.