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| Abstract | This chapter describes the detailed protocol for the isolation and purification of islets of Langerhans from rodent pancreas using collagenase digestion. The first step of the process is to separate and isolate the insulin-producing islets of Langerhans from the rest of the pancreas. The pancreas is excised from the animal, trimmed of nonpancreatic tissues before being inflated and chopped into small pieces. The connective tissue is then broken down with a collagenase enzyme solution to selectively digest the bulk of the exocrine tissue while leaving the endocrine islets intact and separated from their surrounding non-islet tissue. Once this process is completed, the islets of Langerhans are separated from the remaining mixture by centrifugation and purified by the means of hand picking. Once isolated, the subsequent islets can be used for several varied experimental processes, including transplantation, the study of pathophysiological mechanisms in diabetic conditions, and in the screening of novel therapeutic approaches in pharmacological research. | |
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# Chapter 9

# Isolation and Purification of Rodent Pancreatic Islets of Langerhans

Jacqueline F. O'Dowd and Claire J. Stocker

## Abstract

This chapter describes the detailed protocol for the isolation and purification of islets of Langerhans from rodent pancreas using collagenase digestion. The first step of the process is to separate and isolate the insulin-producing islets of Langerhans from the rest of the pancreas. The pancreas is excised from the animal, trimmed of nonpancreatic tissues before being inflated and chopped into small pieces. The connective tissue is then broken down with a collagenase enzyme solution to selectively digest the bulk of the exocrine tissue while leaving the endocrine islets intact and separated from their surrounding non-islet tissue. Once this process is completed, the islets of Langerhans are separated from the remaining mixture by centrifugation and purified by the means of hand picking. Once isolated, the subsequent islets can be used for several varied experimental processes, including transplantation, the study of pathophysiological mechanisms in diabetic conditions, and in the screening of novel therapeutic approaches in pharmacological research.

**Key words** Islets of Langerhans, Insulin, Isolation, Endocrine pancreas, β-cell, Collagenase

## 1 Introduction

The pancreas is a highly vascular retroperitoneal gland located in the abdomen behind the stomach and on the posterior abdominal wall surrounded by the liver and intestine. It is composed of both exocrine and endocrine tissue. Embedded throughout the exocrine glandular tissue, clusters of secretory endocrine cells, called the islets of Langerhans, secrete hormones directly into the bloodstream. Discovered in 1869 by the German pathological anatomist Paul Langerhans, the islets of Langerhans constitute only 1–3% of the total pancreatic volume [1] but fulfill a vital role in glucose homeostasis. The number of islets within a human pancreas can range from 200,000 to almost two million. Each islet itself can range in size from a cluster of a few cells less than 40 μm in diameter to ovoids of 400 μm in diameter [2]. Within the pancreas, islets are not randomly distributed: small islets (160 nm or less, in diameter)

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tend to be scattered throughout the exocrine tissue while larger islets, 250 nm or more in diameter, appear to be located near larger ducts and blood vessels [3].

The ability to isolate islets from the pancreas enables investigators to use them in a number of downstream applications [4]. Once isolated, islets of Langerhans can be maintained as viable units for extended periods of time in tissue culture or they can be used in more acute experiments investigating aspects of mechanistic functionality. Isolated islets have long been used for the static and perfusion incubations (to assess hormone release in response to compounds, but recent advances such as RNA interference (RNAi), a powerful and convenient tool for studying gene function, mean that the ability to isolate islets is a useful tool that allows investigators to gather functional information without using cell lines.

Islets are isolated by a modification of the method originally described by Lacy in 1967 using enzymatic digestion with a relatively crude preparation of collagenase [5]. The less fibrous nature of the rodent pancreas, as compared to man, makes the release of islets easier and allows for the preferential use of less purified collagenase preparations in many laboratories. The first step of the process is to remove the pancreas from the animal and trim it of non-pancreatic tissues. The pancreas is then inflated to increase the surface area and the connective tissue digested with collagenase. After digestion is complete, the mixture is centrifuged to separate the islets from non-islet tissue. The pellet is then resuspended in physiological saline. A small sample of the resuspended pellet is then placed on a black petri dish containing more buffer. The black background of the petri dish allows the white islets to be visible under a dissection microscope with an external light source. Islets are then purified by hand picking using a very fine glass pipette. The picking procedure is repeated twice to ensure no carryover of unwanted exocrine tissue debris and to ensure that the islets are of the highest quality and purity. It is very important that the islets being isolated are intact and relatively pure. Contamination of islets with acinar cells when used for functional studies may lead to a high level of proteases that will later influence islet integrity and functionality during incubation. The highest level of purity is required if protein or RNA is to be extracted from the isolated islets as any contamination of the preparation with acinar cells will cause spurious data to be obtained.

With the increasing focus on the need to isolate viable islets and to overcome the berries of islet transplantation, a number of modifications to this low activity collagenase digestion technique have been described including low temperature Percoll centrifugation [6] and sedimentation of islets [7], as well as their embedding in hydrogel post purification [8].

In this chapter, a method for the isolation of islets of Langerhans from the pancreas by collagenase enzymatic digestion and then harvesting by handpicking individual islets is described. Although the process of handpicking islets is both time-consuming and labor-intensive, this method gives islet preparations with the highest purity.

## 2 Materials

### 2.1 Reagents

1. Collagenase (Type XI, Sigma) (*see* **Note 1**).
2. Buffered saline solution: either Gey and Gey or Krebs Ringer buffer supplemented with 1 mM $CaCl_2$, 4 mM glucose and gassed with $CO_2$:$O_2$ (95:5).
   (a) Gey and Gey Buffer—111 mM NaCl, 27 mM $NaHCO_3$, 5 mM KCl, 1 mM $CaCl_2$, $MgCl_2$, 0.3 mM $MgSO_4$, 1.18 mM $Na_2HPO_4$, 0.29 mM $KH_2PO_4$, and 4 mM D-glucose gassed to pH 7.4 by $CO_2$:$O_2$ (95:5).

   A ten times stock solution without KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$, $KH_2PO_4$, and D-glucose can be made and kept for 1 month at room temperature.
3. RPMI-1640 culture medium.

### 2.2 Equipment

1. Sterilized Forceps.
2. Sterilized scissors: One for skin incision and another for removal of pancreas from adjacent tissue and another for chopping the pancreas into pieces.
3. 10-ml syringe.
4. 25-G needle.
5. 15-ml polypropylene tubes.
6. 25-ml glass measuring beaker.
7. 50-ml conical flask with lid.
8. Water bath with shaker.
9. Plastic-Pasteur pipette.
10. 90-mm petri-dish painted 'in house' with two layers of black enamel paint to ensure a darkened background to visualize and pick islets against.
11. Dissection microscope with external light source.

### 2.3 Islets Isolation

1. For islets isolated from normal rats (150–180 g) use two animals per isolation or three normal mice (*see* **Note 2**).
2. Just prior to dissection, euthanize the donor rodent by an appropriate technique.

3. On a sterilized area, make a V-incision starting at the genital area, move the bowel to the left side of the open rodent. This will expose the pancreas which is tan in color and can be easily differentiated from the surrounding fat as can the spleen.
4. Using forceps to grasp the spleen, gently push up and back the stomach.
5. Cut the pancreas away from the surrounding fat and where attached at the small intestine.
6. Remove the pancreas from the body and place the pancreas, still attached to the spleen, into 100 ml of Gey and Gey in a Petri dish and cut away from the spleen—any retained fat, lymph nodes, and other non-pancreatic tissues.
7. Inflate the pancreas by inserting a 25-gauge needle attached to a 10-ml syringe into the pancreas and injecting small volumes (1–2 ml at a time) of Gey and Gey solution into all the folds of the tissues so that the pancreas increases in size, thus creating a larger surface area.
8. Place the pancreata into a glass 50-ml beaker and chop using scissors until all the pieces were approximately the same size (1 mm × 1 mm).
9. Transfer the contents to a 15-ml tube and centrifuge for 5 min at 100 × *g*. After centrifugation, remove the supernatant, which contains fat, and transfer the pellet to a 15-ml conical tube.
10. Digest the pancreas with collagenase type XI (1–2 mg/pancreas) in a 1:1 mixture of Gey and Gey solution in order to release islets from exocrine tissue.
11. Place the conical tube in a shaking water bath at 37 °C and shake at 800 oscillations per minute until the solution appears "milky" and most of the pieces of pancreas are digested (approximately 5–10 min—time varies with strain and age of the animal). During this time, take a small amount (approximately 400 μl) in a plastic pipette and examine under a dissecting microscope to confirm whether islets are free from the exocrine tissue. If there are no islets visible continue the shaking by hand and repeat the process (*see* **Note 3**). Normally, a total shaking time of 6–8 min is enough to release the bulk of free islets.
12. Place the contents of the conical flask and stop the digestion by adding 15 ml of cold buffer.
13. Centrifuge at 500 × *g* for 5 min to remove the collagenase in the solution.
14. Aspirate the supernatant and resuspend the pellet in 15 ml of the isolation buffer.

15. Add a sample of the resuspended pellet (1 ml) to a blackened 90-mm Petri dish, add buffer to the top of the Petri dish, and mix with a plastic Pasteur pipette.
16. Using a dissection microscope and an external light source, handpick and count individual islets using a drawn out glass pipette (*see* **Note 4**).
17. Only pick clean and intact islets free of exocrine tissues (*see* **Note 5**).
18. Repeat the picking procedure at least twice.
19. Incubate the islets with isolation buffer in a 37 °C water bath until use. Always use the islets within 30 min to 1 h after isolation.
20. While the yield of islets is variable, approximately 600–1200 islets are obtainable from two Wistar rats and 400 from three mice using this method (*see* **Note 6**).

### 2.4 Islets Culturing

For certain experimental protocols, it may be necessary to culture the isolated islets. When culturing, it is important to handle the islets under aseptic conditions and use sterile tubes and flasks.

1. Wash the islets in more than ten volumes of sterile Gey and Gey solution.
2. Resuspend groups of 200 islets in RPMI-1640 containing 11.1 mM glucose (*see* **Note 7**) and transfer to a six-well cell-culture plate.
3. Incubate the islets in a humidified culture incubator with 5% $CO_2$; 95% air.

## 3 Notes

1. A wide variety of collagenase preparations are available for islet isolation but in our experience Collagenase Type XI from Sigma-Aldrich is best suited for isolations. This crude mixture isolated from *Clostridium histolyticum* contains several enzymes (collagenase, neutral proteases, clostripain, and caseinase) that act together to break down tissue. While collagenase Type XI has one of the highest collagenase activities, not every batch is equivalent, and it is usually appropriate to test small quantities from several different batches before choosing to make a bulk purchase of a particular batch.
2. The age and weight of the animals can influence the numbers of islets of Langerhans obtained. Rats heavier than 180 g often yield fewer islets per pancreas. Although younger animals can give high yields, the islets obtained from these animals should be considered from a 'juvenile' stage of development.

3. If no islets are isolated or isolated islets are not intact, the islets are over-digested. This can be prevented by decreasing the collagenase digestion time or collagenase concentration (*see* **step 10**).
4. Normal bore pipettes draw up too much liquid as islets are picked, so drawn pipettes are preferential. Draw glass pipettes by heating in a Bunsen flame. Once the glass begins to give, pull sharply to yield pipettes with a bore of approximately 1 mm.
5. If most of the islets are not discrete and difficult to isolate (*see* **step 15**), the exocrine tissues are under-digested. In order to overcome this problem, increase the collagenase digestion time (*see* **step 10**) and/or increase the collagenase concentration (*see* **step 10**) and ensure vigorously shaking of the vial to disrupt the pancreas (*see* **step 11**).
6. The yield of islets will vary depending on the age and strain. Diabetic and older animals will have reduced numbers of viable islets. Digestion continues as the handpicking selection proceeds; therefore, speed is important. Islets should be collected within 15–20 min, and the entire isolation completed within 45 min to 1 h of removal of the organ.
7. The culture medium can be altered depending on the experimental conditions required.

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