What is an acceptable rodent islet yield?

Survey summary of islet yields by age, sex, and strain of the animal

VitaCyte®

White Paper

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Overview

Isolation of rodent islets is a unique skill that usually requires a person interested in adopting this skill to receive advice or training from an islet isolation expert who will provide a demonstration of the method but will also share often unwritten recommendations that are often critical to the success of the procedure. A complication arises when either individual is faced with isolating islets from a new and different rodent strain. The key question asked is what islet yields should I expect? It has been known for many years that islet yields vary by the age, sex, and strain of the animal. These expected islet yields are rarely published in the scientific literature and if mentioned, they are briefly noted in the materials and methods or in a table in the results section. Commonly, the primary way an individual obtains this information is to ask their colleagues about their islet isolation experience using animals with similar characteristics: age, sex, and strain.

To provide a resource to the rodent islet isolation community, VitaCyte surveyed its users and asked them to submit rodent islet yields isolated from different strains of mice and rats. These results are restricted to those isolations performed using purified collagenase and protease for reasons outlined below. If you want to contribute your results to this ongoing survey, please complete the form on the last two pages of this document and send a scanned copy back to feedback@vitacyte.com. Title the email rodent islet strain survey and your results will be added to the table. The source of this information will be kept confidential.

Overview

Many factors contribute to variable yields of rodent pancreatic islets recovered from different strains of animals. One important factor is the age, weight, sex, and strain of the animal[1,2]. Islet yields for different strains of animals is not easily found nor mentioned in reviews on this topic[3]. To address this information gap, VitaCyte surveyed its customers who perform rodent islet isolations and summarized these data in two separate tables below, one for mouse, the other for rat.

Presentation of survey results

Each table includes detail of the rodents used in the isolation procedure, islet yield per pancreas, the methods used to isolate the islets. We only included those data which used purified collagenase and

[1] Bock T, Pakkenberg B, and Buschard K (2005) Genetic Background determines the size and structure of the endocrine pancreas. *Diabetes* 54:133-137

[2] Bonner-Weir S (2000) Islet growth and development in the adult. *J Mol Endocrinol* 24:297-302

[3] Carter JD, et al (2009) A practical guide to rodent islet isolation and assessment. Biological Procedures Online 11:3-31.

protease for rodent islet isolation. Some may object to this restriction but there are several reasons for this decision.

First, each lot of a crude or enriched collagenase is unique since it reflects the biochemical composition of the *Clostridium histolyticum* culture supernatant used to generate the product[4]. These products are minimally purified and contain multiple forms of collagenase, multiple neutral proteases with different protease activities, other enzymatic activities, biopigment (responsible for the color of the product), and variable amounts of endotoxin. To address this heterogeneity, manufacturers separated these products into different Types which were categorized by different enzyme activities. These activities appeared to correlate with the success of isolation of specific types of cells but there is an overlap of enzyme activities across different types of crude collagenase[4].

Second, selection of collagenase is recognized as an important variable in the quality of mammalian islets recovered from the pancreas. Many labs that want to control this variable will carefully screen different lots of collagenase before making the final selection and then purchasing a large amount of product that lasts for several years. The effort required to select a "good" lot of crude or enriched collagenase for long term use illustrates the lack of lot consistency normally found in these products

And last, if the islet recovery data is to benefit the scientific community, then the quality of collagenase and protease used to recover islets must be defined. Several manufacturers, including VitaCyte, make purified collagenase-protease enzyme mixtures that are used for rodent islet isolation. Since these products contain purified collagenase and purified neutral protease, the biochemical composition and the specific activity of these enzyme mixtures are fixed to ensure reproducible manufacture of these products.

Reliability of summarized data

The data summarized in the table below is only as good as the source and amount of data contributed to the survey. We encourage your contribution to this data summary. If you want to submit your isolation results, please complete the survey form on the last two pages of this document and send an electronic or scanned copy of the document to feedback@vitacyte.com. We will include all results we receive, independent of the manufacturer of the purified enzyme product, in updated versions of this white paper and on VitaCyte's website where these data can be found within the summary page on "Rodent Islet Applications Overview" (see https://www.vitacyte.com/product-applications/rodent-islet-applications-overview/) . The source of this information will be kept confidential. For the reasons stated above, we will not include data from rodent islets isolated using crude or enriched collagenase products where there has been minimal biochemical characterization of the product.

[4] VitaCyte Whitepaper: Lot Qualification: A common but costly pathway to select enzymes for cell isolation. What are the known unknowns when using most collagenase products? Available on request, send e mail to feedback@vitacyte.com

Results from mouse islet isolation survey using purified tissue dissociation enzymes

Strain (Supplier) Age & Weight	Sex M (♂) F(♀) Both	Avg islet yield per pancreas	Method used[1] (agitation?[2])	Product (Mfg) Enzyme mass or activity / mL	Digest Time min	Purification Ficoll or Histopaque (Density g / mL)
C57/BL6 (JAX) >6 mo > 40 g	B	400-1000	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 (JAX) 6-8 wk 20-25 g	B	200-300	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 (JAX) 3-4 wk 20-25 g	B	150-200	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 (JAX) 2 wk	B	100-150	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 (JAX) 1 wk	B	50-100	Modified Lacy[4] (mechanical)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 (JAX) high fat feeding 20-40 wk 50 g	M	800-1500	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
C57/BL6 8-10 weeks	M	100	Gotoh (gentle mechanical	TL (Roche) 1.576 Wunsch U/mL	7-8	Ficoll gradient (Corning)
DBA (JAX) 8 wk	M	400	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
DB/DB (JAX) 8 wk	F	700	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
NMRI 11-12 wk	F	466	Gotoh (static)	TL (Roche) 0.125 mg/mL	16	Histopaque (1.1 g/mL)
NOD (JAX) >12 wk	B	< 50	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
NOD (JAX) 8-10 wk	B	100-120	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
NOD (JAX) 4 wk	B	150-200	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
NOD/SCID (JAX) 8 wk 25 g	B	300	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)
OB/OB (JAX) 8 wk	F	500	Gotoh(static)	RI (VitaCyte) 12,500 CDA U/mL 2500 NPA U/mL	17	Corning LSM (1.077 g/mL)

Results from rat islet isolation survey using purified tissue dissociation enzymes

Strain (Supplier) Age & Weight	Sex M (♂) F(♀) Both	Avg islet yield per pancreas	Method used[1] (agitation[iii][2]?)	Product (Mfg) Enzyme mass or activity / mL	Digest Time min	Purification Ficoll or Histopaque (Density g / mL)
Lewis (Harlan) 6-8 wk 200g	B	1000	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	20.5	Corning LSM (1.077 g/mL)
Lewis (Harlan) 4 wk 100g	B	800	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Sprague Dawley 350-400g	M	500	Gotoh(static)	RI (VitaCyte) 8,333 CDA U/mL 1667 NPA U/mL	12	
Sprague Dawley 350-400g	M	500	Gotoh (mechanical shaking 60 RPM)	RI (VitaCyte) 8,530 CDA U/mL 1,704 NPA U/mL	12	Corning Ficoll gradient
Sprague Dawley (Taconic) 6 mo 350-400 g	B	1000	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	20.5	Corning LSM (1.077 g/mL)
Sprague Dawley (Taconic) 6-8 wk 200 g	B	1000	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Sprague Dawley (Taconic) 4 wk 100 g	B	800	Gotoh (gentle mechanical	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Sprague Dawley (Taconic) 2 wk	B	300	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Sprague Dawley (Taconic) 1-7 d	B	150-200	Modified Lacy (mechanical)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Sprague Dawley (Taconic)	F (Preg)	1500	Gotoh (static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
Wistar (Charles River) 1 mo 100 g	M	100	Gotoh (mechanical)	TL (Roche) 68 ug/mL	28	Histopaque 1077
ZDF >500 g	M	20-40	Gotoh (mechanical shaking 60 RPM)	RI (VitaCyte) 8530 CDA U/mL 1704 NPA U/mL	12	Corning Ficoll gradient
ZDF 370 (Charles River) 10 wk		200-400	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)
ZDF 371 (Charles River) 10 wk		500	Gotoh(static)	RI (VitaCyte) 8333 CDA U/mL 1667 NPA U/mL	19.5	Corning LSM (1.077 g/mL)

Abbreviations: CDA = collagen degradation activity, LSM = Lymphocyte separation media, NPA = neutral protease activity, U = Units

1 -Gotoh method perfuses enzyme solution in situ in the pancreas, after inflation of the organ, the pancreas is removed and incubated in a water bath
-Lacy method perfuses Hanks BSS (HBSS) in the organ, after inflation, organ removed, minced, then incubated with enzyme solution
-Modified Lacy procedure where no in situ infusion of HBSS, pancreas removed, minced, and in the middle of the digest, tubes shaken by hand for 30 seconds

2 -Static digestion = leaving enzyme in organ with no mechanical agitation until last step of digest
-Mechanical means agitating organ or minced tissue during digestion either using manual shaking, mechanical wrist shaker, stir bar, or agitation in using shaking water bath or instrument (e.g., Rotator-Genie™).

Survey: Strain effects on rodent islet yield

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Why VitaCyte needs this information

- VitaCyte supports increasing research productivity of scientists who use rodent islets for diabetes research.
- Common question when using a new rat or mouse strain is how many islets should I recover? Difficult to find this information.
- The questionnaire below asks for data from your laboratory where either purified or defined enriched collagenase (e.g., DE Collagenase) is used in the islet isolation procedure.
- Collection of these data will enable others to compare their isolation results and reagents to others
- Islets recovered after digestion with crude or enriched collagenase (e.g., Sigma Types V or VIII, Sigma Type XI, Collagenaase P) are not included because of lot to lot variability
- Survey results will be updated on a quarterly basis

To complete the questionnaire:

- Complete the information on the next page
- Use one letter abbreviation for mouse (M) or rat (R) and male (M), female (F) or both (B)
- It is assumed that the islet yield is expressed as an islet count
  - If it is expressed as an islet equivalent count (IEQ) where the volume of islet mass is used to express the count, please note this in the comments
- It is important to capture the amount or activity of the enzyme you routinely use for perfusion into the organ or digestion of minced pancreatic tissue
- Provide your contact information in the form to the right

Your contact information

Name:

Institution:

Department/Lab PI:

E mail:

Phone:

- Send a scanned copy of the completed survey to feedback@vitacyte.com or fax to 317-917-3459 and title the message "Rodent islet survey contribution"

Additional Comments

Thank you for

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	Species **M**ouse or **R**at	**Strain**	**Animal Supplier (if available)**	**Sex** **M**ale **F**emale **B**oth	**Age and/or weight**	**Ave islet yield per pancreas**[1]	**Comments**
1							
2							
3							
4							

1. If yield is expressed other than islet count (i.e. Islet equivalent count), please note this unit in the comment section

	Manufacturer enzyme product	**Product name/ catalog number**	**Amount/Units of enzyme per mL of final solution**[2]	**Method Gotoh**[3] **or Lacy**[4]	**Static or Mechanical digestion**[5]	**Time Digestion (min)**
1						
2						
3						
4						

2. Usually expressed as mg or g; or Wunsch units (WU); or collagen degradation activity (CDU or CDA U)
3. Gotoh method perfuse enzyme solution in situ in the pancreas, after inflation of the organ, the pancreas is removed and incubated in a water bath
4. Lacy method perfuse Hanks BSS in the organ, after inflation, organ removed, minced, then incubated with enzyme solution
5. Static digestion = leaving enzyme in organ with no mechanical agitation. Mechanical means agitating organ or minced tissue during digestion either using manual shaking, stir bar, or agitation in using shaking water bath or instrument (e.g., Rotator-Genie™). Please note specific agitation method used.