Version 2

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Human Islet Quantification and Purity Assessment V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bt57nq9n

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ABSTRACT

This protocol describes the Quantification and Purity of Assessment of human islets, as performed by the Alberta Diabetes Institute IsletCore. www.bcell.org/adi-isletcore

DOI

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PROTOCOL CITATION

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WHAT'S NEW

Minor edits and new examples, for clarity.

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49055

MATERIALS TEXT

MATERIALS

Dimethyl Sulfoxide **Fisher Scientific Catalog #D128**

Dithizone **Sigma-aldrich Catalog #43820**

0.45um Syringe Filter **Fisher Scientific Catalog #09-740-116**

BEFORE STARTING

HBSS is prepared as described in Human Islet Isolation Media protocol.

Preparation and use of Dithizone stain in Human Islet Preparations

1 Preparation of DMSO-dithizone (DTZ)

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 color.
3. Alternatively: 50µl of islet suspension, 50µl dithizone, and 2000µl HBSS.

Islet Sampling

2 Islet samples are prepared as described in Human Islet Sampling and Human Islet Isolation protocols.

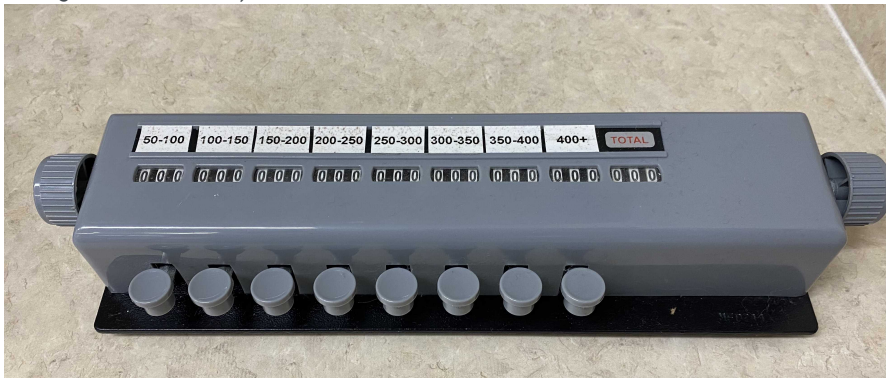
Human Islet quantification - Islet Equivalent (IEQ) counts

3 Add ~1ml DTZ to sample and incubate until islets are visibly stained red. Add ~1ml HBSS to dilute staining background if necessary.

4 Place sample on stage and determine IEQ (single sample counted in duplicate) using the following steps.

5 Using the ocular with graticule (1 square = 100µm x 100µm), measure the diameter (or circular equivalent) of each particle in the sample and tabulate in the corresponding column. (Refer to the table in step 11). *Islet particles <50µm are not included.*

6 Once the entire sample has been counted, calculate the sub-totals for each column and total of all columns and enter values into the table in step 11. To facilitate easier counting, use a 9-channel benchtop cell counter (Bal Supply - Catalogue number 808CI).



7 The multiplication factor (table, step 11) is determined by dividing the total volume by the sample volume. *eg. 100mL / 50µL = 2000X (100/0.050=2000)*

Assessment of Islet purity

Citation: James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald (04/30/2021). Human Islet Quantification and Purity Assessment. https://dx.doi.org/10.17504/protocols.io.bt57nq9n

8 Percent purity is recorded by estimating the ratio of islets to exocrine tissue. For example, if the area of islets is equal to the area of exocrine tissue, the purity would be 50%.

Assessment of the percentage of trapped or mantled islets

9 Percent trapped is determined by estimating the ratio of trapped versus total islets

Assessment of Islet Morphology

10 Visually assess the morphology of the islets and enter a score in the Islet Scoring Table. For example, if the islets are round and spherical enter a value of 2 for "shape". If overall the islet borders are well-rounded enter a value of 2 under "border". If the integrity of the islets are in-between (not fragmented and not solid/compact), enter a value of 1 under "integrity". If there a few small dithizone-stained islets of less than 25µm in diameter, then mark a 1 under "Islets <25µm". And finally, if there almost no clumping of groups of islets, then mark a value of 2 under"clumping". When the scores are totaled the final islet score is 8.

Islet scoring table

A	B	C	D	E	F	G	H	I
Shape (3D)	**Border**	**Integrity**	**islets <25µm**	**clumping**				
Flat/planar - 0	irregular - 0	fragmented - 0	many - 0	many - 0				
in between - 1	in between - 1	in between - 1	a few -1	a few -1				
spherical - 2	well-rounded - 2	solid/compact - 2	almost none - 2	almost none - 2	**Total score**	**Purity (%)**	**Trapped (%)**	

11 Islet Equivalent Determination

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A	B	C	D	E	F
Date	(dd/mmm/yyyy)				
Suspension volume (mL)	100				
Sample volume (ml)	0.05				
Multiplication factor	2000				
purity					
IEQ size range (µm)	**Conversion factor to correct to 150µm**	**Count 1**			**Corrected Mean**
50-100	0.1685	1a	2a	(1a+2a)/2	Mean * 0.1685
100-150	0.685	1b	2b	(1b+2b)/2	Mean * 0.685
150-200	1.685	1c	2c	(1c+2c)/2	Mean * 1.685
200-250	3.5	1d	2d	(1d+2d)/2	Mean * 3.500
250-300	6.315	1e	2e	(1e+2e)/2	Mean * 6.315
300-350	10.352	1f	2f	(1f+2f)/2	Mean * 10.352
350-400	15.833	1g	2g	(1g+2g)/2	Mean * 15.833
				IEQ in sample	Total of corrected means = sum of above cells
				Total IEQ	Total of corrected means * multiplication factor

11.1 Example of Islet Equivalent Determination

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A	B	C	D	E	F	G	H	I	J	K	L	M	N
Example isolation													
Date	01-Jan-2000												
Suspension volume (mL)	100												
Sample volume (ml)	0.05												
Multiplication factor	2000												
IEQ size range (µm)	**Conversion factor to correct to 150µm**	**Count 1**	**Count 2**	**Mean Count**	**Corrected Mean**								
50-100	0.1685	59	64	61.5	10.36								
100-150	0.685	25	23	24	16.44								
150-200	1.685	5	6	5.5	9.27								
200-250	3.5	6	5	5.5	19.25								
250-300	6.315	1	2	1.5	9.47								
300-350	10.352	1	0	0.5	5.18								
350-400	15.833	0	0	0	0								
				IEQ in sample	69.97								
				Total IEQ	139,940								

Determination of the number of Islet Particles (IP) and the Islet Particle Index (IPI)

12 First, determine the total number of islet particles (IP).

From the above table, total all the values in the "Mean Count" column and then multiply this value by the multiplication factor.

example (from above). - (61.5 + 24 + 5.5 + 5.5 + 1.5 + 0.5) * 2000 = 197,000 IP

Next, calculate the islet particle Index by Dividing the number of Total Islet Equivalents by the number of Islet Particles

example (from above) - 139,940/197,000 = 0.71 IPI

The significance of the Islet Particle Index is that an IPI of greater than 1 represents a population of islets greater than 150µm in diameter. Conversely, an IPI less than 1 indicates a population of islets less than 150µm.

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