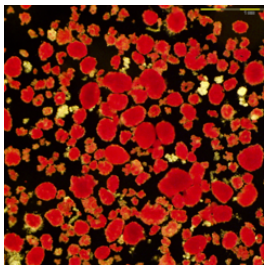
Jan 25, 2019 Version 1

Human Islet Quantification and Purity Assessment V.1



Endocrinology

In 2 collections

DOI
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Human Cell Atlas Metho... CIRTNR2FIC 1 more workspace

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Protocol status: Working
We use this protocol and it's working

Created: August 10, 2018

Last Modified: January 25, 2019

Protocol Integer ID: 14651

Materials

MATERIALS

- Dimethyl Sulfoxide **Fisher Scientific Catalog #**D128
- Dithizone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43820
- 0.45um Syringe Filter **Fisher Scientific Catalog #**09-740-116

Before start

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 colour.
3. Alternately, 100µl of islet suspension, 100µl dithizone and 200µl HBSS.

Preparation and use of Dithizone stain in Islet Preparations

2 Islets samples are prepared as described in **Human Islet Sampling** and **Human Islet Isolation** protocols.
DMSO-Dithizone is prepared as described in **Human Islet Isolation Media** protocol.

Preparation and use of Dithizone stain in Human Islet Preparations

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 gradacule (1 square = 100µm x 100µm), measure the diameter (or circular equivalent) of each particle in the sample and tabulate in corresponding column. (Refer to table in step 9). *Islet particles <50µm are not included.* Once entire sample has been counted calculate the sub-totals for each column and total of all columns and enter values into table in step 9.

6 The multiplication factor (table, step 9) is determined by dividing the total volume by the samp*le volume. Eg. 100mL / 50µL = 2000X (100/0.050=2000)*

7 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 exocrine tissue, the purity would be 50%.

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

9 Visually assess the morphology of the islets entering a score in the Islet Scoring Table. For example, if the islets are round with a solid border and with a dense compact overall look, the score would be 2 out of 2 for each category.

Islet scoring table

	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 (%)**	

10 Islet Equivilent Determination

	Date								
	Suspension volume (mL)	100							
	Sample volume (ml)	0.05							
	Multiplication factor	2000							
			Number	Corrected	Total				
	IEQ range	Conversion	Count 1	Count 2	mean	Count 1	Count 2	Mean	I.E.Q.
	50-100	0.1685							
	100-150	0.685							
	150-200	1.685							
	200-250	3.5							
	250-300	6.315							
	300-350	10.352							
	350-400	15.833							

						Count 1	Count 2	Total	
				Total					