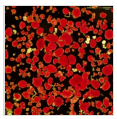

Human Islet Quantification and Purity Assessment

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Dimethyl Sulfoxide	D128	Fisher Scientific
Dithizone	43820	Sigma-aldrich
0.45um Syringe Filter	09-740-116	Fisher Scientific

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 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					

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