

Protocol for Pancreatic Islet Quantification and Purity Determination

Introduction:

Insulin producing cells are rich in zinc and stain with Diphenylthiocarbazone (DTZ), thus aiding in distinguishing islets from non-islet tissue. Islets are scored based on size and the number is normalized using a calculation to provide the number of islets equivalent to a nominal 150µm.

Procedure:

1. The sample should contain 100-300 islets for each duplicate count.
2. Place the sample in a 60mm Petri dish with grids. Record the sample volume on the pancreatic Islet Quantification/Purity Worksheet
3. Add enough culture medium to cover the bottom of the container and allow the islets to be evenly dispersed.
4. Add approximately 0.3-0.5 ml of DTZ working solution to each count container and allow to stain for approximately 30-60 seconds.
5. Place the count petri dish on the microscope stage on a stage adapter. The islets are counted one grid at a time to ensure all islets are counted and none are inadvertently re-counted or missed.
6. Count all islet (DTZ positive) and non-islet (DTZ negative, including DTZ negative islets) tissues with a diameter of approximately 50µm or greater. Using the micrometer in the eyepiece estimate the islet size range. A minimum of 100 islets should be counted for each replicate. Record the individual counts for each of the categories for islets and non islet fragments (size not recorded for DTZ negative cells) on the islet quantification/Purity Worksheet.
7. Add the counts in each category for each count sample (replicate) and record the value in the space provided on the islet Quantification/Purity Worksheet. Calculate the average for total islets, each category and the DTZ negative cells.
8. Calculate Total number of islets in the sample by using the average total count in each category using the following equation:

$$\text{Total Islet number} = \text{Average Total count} \times \frac{\text{Total Volume(ml)}}{\text{Count volume(ml)}} \times \text{Dilution Factor}$$

Calculate Total Islet Equivalents in the sample by using the average total count in each category with the following equation:

$$\text{Total Islet Equivalents} = \sum \left(\begin{array}{l} \text{(Avg.Cat.1x0.125)} \\ \text{(Avg.Cat.2x1)} \\ \text{(Avg.Cat.3x4.63)} \\ \text{(Avg.Cat.4x12.7)} \\ \text{(Avg.Cat.5x 20.8)} \end{array} \right) \times \frac{\text{Total Volume(ml)}}{\text{Count volume(ml)}} \times \text{Dilution Factor}$$

Calculate the Islet Index in the sample by using the following equation:

$$\text{Islet Index} = \frac{\text{Total Islet Equivalents}}{\text{Total Number of Islets}}$$

Calculate the islet purity by using the following equation:

$$\% \text{ Purity} = \frac{\text{Average DTZ Positive}}{\text{Average DTZ positive} + \text{Average DTZ negative}} \times 100\%$$

Pancreatic Islet Quantification/Purity Worksheet - Calculation of IEQ

Islet ID:		-						-		
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Sample description:

1. Pre-Purification
1. Post- Purification
2. Post Isolation Overnight: _____ Hours
3. Day of Shipping
4. Other: _____

Count Vol: _____ ml Total Vol: _____ ml Dilution Factor _____

Reagent:

Reagent	Lab Notebook Ref	Preparation Date	Expiration Date
DTZ			

Equipment:

Microscope ID	Serial number	Last serviced (m/yy)	Service Due (m/yy)	Last Calibrated (m/yy)	Calibration Due (m/yy)

Counts:

Diameter	Raw count(s)	Average Count	Conversion Factor	Avg. Islet Count X Conv. Factor
50-100µm			0.125	
100-200µm			1.000	
200-300µm			4.630	
300- 400µm			12.70	
>400µm			20.8	
Total				
DTZ positive				
DTZ Negative				

Total IEQ _____

Total Islet Number _____

Islet Index: _____

%Purity _____ %

Comments:

- Full Count
- Estimate

Performed By: _____ Date: _____

Reviewed By: _____ Date: _____