



Assessment of Islet Viability by Fluorescent Dyes

PURPOSE: A procedure for the assessment of intact pancreatic islet viability.

MATERIALS:

Reagents:

- Fluorescein Diacetate (FDA), Sigma, # F7378
- Propidium Iodide (PI), Invitrogen, # P1304MP
- Phosphate Buffered Saline (PBS), Mediatech, #21-030-CV

Equipment (or equivalent):

- Fluorescence Microscope, Zeiss Axiovert 200
- Zeiss AxioCam Digital Camera
- 12 x 75mm snap cap, Falcon Tubes, BD Falcon 2054
- Microfuge tubes
- Pipettor, Pipet tips
- Petri Dishes, Falcon 25373-085

SAFETY: Always wear gloves and observe standard chemical procedures.

- Fluorescein Diacetate: Avoid contact and inhalation.
- Propidium Iodide Solution: Protect from light. Product may be toxic if inhaled, swallowed, or splashed on skin. The site of action is the DNA.
- Acetone: Solvent/Flammable. Keep away from heat, spark, and open flame. Keep container tightly closed. Use with adequate ventilation. Avoid contact with eyes. Avoid prolonged or repeated breathing of vapor. Avoid prolonged or repeated contact with skin.

PROCEDURE:

Preparation of Working Reagents:

- Propidium iodide (PI): Prepare a propidium iodide stock [0.5 mg/ml] in PBS. Shelf life is 6 – 12 months when stored at 4°C. Protect from light. Dilute 50 µl with 450 µl PBS and use within 30 min of dilution.
- Fluorescein Diacetate (FDA): Dissolve 100 mg in 20 ml of acetone. Store in 0.25 ml aliquots at -20°C in the dark. Shelf life is 3 months. Dilute 10 µl of this stock with 990 µl of PBS and use within 30 min of dilution.

Staining Procedure:

1. Obtain approximately 150-200 islet particles from a flask and place in a Falcon tube.
2. Allow the islets to settle by gravity and carefully remove the supernatant leaving ~ 0.2 ml.
3. Add 20 µl 1:10 PI stock followed by 20 µl 1:100 FDA to the sample of islets (specifically in that order).
4. After 30 seconds of adding the stain, place in a 35 mm petri dish containing 2.0 ml of PBS. Place the sample on the fluorescence microscope, visualize and photograph utilizing the filter block for fluorescein (em ~ 530 nm) and rhodamine (em >600 nm). Dead cells will stain red; viable cells will stain green.



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5. Also visualize and photograph the sample using bright field illumination. In situations where the purity is low, bright field illumination is necessary to discriminate islet from non-islet particles. Islets will in general appear round or egg-shaped and are more opaque when compared to the pail yellow acinar tissue.
6. Photograph at least 5 separate fields at a total magnification of 100x.

Assessment of Percentage of Viable Cells in Each Islet:

Note: Islets are aggregates of cells, therefore, it is expected that some cells within an individual islet will stain green and some will stain red. The objective of the test is to assess, as accurately as possible, the percentage of *islet cells* that are viable.

Assign cells to categories as follows:

- 0 Few or no cells are green and the majority are red; Average viability = non-viable
- 1 Approx. 75% of the cells are red; Average viability = 25%
- 2 Approx. 50% of the cells are red; Average viability = 50%
- 3 Approx. 25% of the cells are red ; Average viability = 75%
- 4 Few or no cells are red; Average viability = 100%

Calculations:

Total Viable = 0.25. (Sum of Category 1) + 0.5. (Sum of Category 2) +0.75. (Sum of Category 3) + (Sum of Category 4)

Total Number of Islets Counted = (Sum of all Categories)

Percent Viable = (Total Viable X 100)/Total Number of Islets

REFERENCES:

- Acridine Orange/Propidium Iodide Viability Assay, University of Minnesota Diabetes Institute, Minneapolis MN
- Assessment of Pancreatic Islet Viability - Ethidium bromide/Fluorescein Diacetate, Neocrin Company, Irvine, CA

Islet Viability Assessment Worksheet

Isolation Code: _____	VIABILITY CATAGORY				
Sample No.	0 0% Viable (majority red)	1 25% Viable (~ 75% red)	2 50% Viable (~50% red)	3 75% Viable (~25% red)	4 100% Viable (few red*)
FDA examples (100x mag.)					
10x PI examples (100x mag.)					
1					
2					
3					
4					
5					
6					
7					
8					
Total Count					

Issued By _____ Date _____ Form Log Number _____

Islet Viability Assessment Worksheet

CALCULATIONS:

$$\text{Total Count} = [0] + [1] + [2] + [3] + [4] = [\quad]$$

$$\text{Total Viable} = (0.25 \times [1]) + (0.50 \times [2]) + (0.75 \times [3]) + [4] = [\quad]$$

$$\text{Percent (\%) Viable} = (\text{Total Viable} \times 100) / (\text{Total Count}) = [\quad]$$

PERFORMED BY: _____ **DATE** _____

REVIEWED BY: _____ **DATE** _____

- * (G) = Green
- * (R) = Red

Issued By _____ Date _____ Form Log Number _____
