Purified Human Pancreatic Islet: Viability Estimation of Islet Using Fluorescent Dyes, Attachment I, Preparation of Fluorescein Diacetate and Propidium Iodine Solutions – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium

The NIH CIT Consortium Chemistry Manufacturing Controls Monitoring Committee:

The NIH CIT Consortium


Massachusetts General Hospital: S. Deng, J. Lei, J.F. Markmann


NIDDK: T.L. Eggerman


University of Illinois, Chicago: B. Barbaro, J. Martellotto, J. Oberholzer, M. Qi, Y. Wang


University of Wisconsin: L. Fernandez, D.B. Kaufman, L. Zitur

Uppsala University: D. Brandhorst, A. Friberg, O. Korsgren

Supported by grants from the National Institute of Allergy and Infectious Diseases and the National Institute for Diabetes and Digestive and Kidney Diseases.

• At Emory University, U01AI089317.
• At Northwestern University, U01AI089316.
• At the University of Alberta, Edmonton: U01AI065191.
• At the University of California, San Francisco, U01DK085531.
• At the University of Illinois, Chicago, 5U01DK070431-10.
• At the University of Iowa, U01DK070431.
• At the University of Miami, U01DK070460.
• At the University of Minnesota, U01AI065193.
• At the University of Pennsylvania, U01DK070430.
• At Uppsala University, U01AI065192.

In addition, the study was supported by the following GCRC and CTSA awards:

• At Emory University: UL1TR000454.
• At Northwestern University: 5UL1RR025741 and 8UL1TR000150.
• At the University of California, San Francisco, UL1TR000004.
• At the University of Illinois, Chicago, UL1TR000050.
• At the University of Miami: 1UL1TR000460.
• At the University of Minnesota: 5M01-RR000400 and UL1TR000114.
• At the University of Pennsylvania: UL1TR000003.

Address correspondence to: Camillo Ricordi MD, Chairman, CIT Steering Committee, ricordi@miami.edu

To cite this article
Purified Human Pancreatic Islet: Viability Estimation of Islet Using Fluorescent Dyes, Attachment I, Preparation of Fluorescein Diacetate and Propidium Iodine Solutions – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium

CellR4 2015; 3 (1): e1374
Purified Human Pancreatic Islets, Viability Estimation of Islets Using Fluorescent Dyes, Attachment I, Preparation of Fluorescein Diacetate and Propidium Iodide Solutions

1.0 Stock Fluorescein Diacetate (FDA) Solution (24 μM)

Calculation:
FDA FW = 416.4
Stock Concentration = 24 μM
Volume required = 200 mL
FW X Concentration X Volume = (416.4) X (24 x 10⁻⁶) X (200 x 10⁻³) = 0.00199 g FDA

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity to be Added</th>
<th>Quantity Added</th>
<th>Supplier &amp; Lot Number</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA</td>
<td>0.00199 g</td>
<td>g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>200 mL</td>
<td>mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Store Stock FDA solution at ≤ -20°C for up to six months.
- Cover it with aluminium foil, as the dye is light sensitive.
- Label with assigned lot number, date of expiration and initials of preparer.

Assigned Lot Number: __________________________

Prepared by: __________________________ Date: ________________

Reviewed by: __________________________ Date: ________________

2.0 Stock Propidium Iodide (PI) Solution (750 μM)

Calculation:
PI FW = 668.4
Stock Concentration = 750 μM
Volume Required = 25 mL
FW X Concentration X Volume = (668.4) X (750 x 10⁻⁶) X (25 x 10⁻³) = 0.0125 g PI

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity to be Added</th>
<th>Quantity Added</th>
<th>Supplier &amp; Lot Number</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>0.0125 g</td>
<td>g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPBS</td>
<td>25 mL</td>
<td>mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Store Stock PI solution at 2° to 8°C for up to six months.
- Cover it with aluminium foil, as the dye is light sensitive.
- Label with assigned lot number, date of expiration and initials of preparer.

Assigned Lot Number: __________________________

Prepared by: __________________________ Date: ________________

Reviewed by: __________________________ Date: ________________