**SOP:** **Isolation and culture of Pancreatic Islets**
**Date modified:** **August 08, 2009**
**Modified by:** **Jeremy Simon (Lieb Group)**

## Source Information:

Human cadaver. Isolated as in:

Bucher, P. et al. Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. *Transplantation* **79**, 91-7 (2005).

## Notes:

All experiments were performed according to protocols approved by the Institutional ethical committees of the Hospital Clinic de Barcelona, Geneva University Hospitals, Ospedale San Raffaele di Milano, and Hospital de Bellvitge.

## Materials List:

1. Neutral protease NB (SERVA Electrophoresis)
2. Collagenase NB1 (SERVA Electrophoresis)
3. Hanks' Balanced Saline Solution (Mediatech, Herndon, VA)
4. Pefabloc SC Plus (Roche)
5. HEPES (Mediatech, Herndon, VA)
6. Biocoll gradients (Biochrom KG, Berlin, Germany)
7. COBE 2991 cell processor (COBE, Lakewood, CO)
8. 10mg Dithiazone (Sigma)
9. DAPI, α-insulin, α-glucagon antibodies
10. Fluorescein diacetate (Sigma)
11. Propidium iodide (Sigma)

## Enzyme Solution (final neutral protease activity of 30 DMC U):

20 dimethylcasein units (DMC U) Neutral Protease NB
1 vial (498mg) collagenase NB1 (2,000 PZ units)
300mL Hanks' Balanced Saline Solution
Pefabloc SC Plus (final concentration of 4mM)
HEPES (final concentration of 25mM)

**Procedure:**

**A. Digestion of pancreas and isolation of islet cells**

1) Perfuse the pancreas with 300mL cold enzyme solution, using controlled pump perfusion, in a digestion chamber at 37ºC
2) Purify the collected islets in continuous Biocoll gradients using a COBE 2991 cell processor

**B. Assessment of islet purity**

1) Analyze islet morphology by light microscopy
2) Assess islet purity and number with dithizone staining using a small aliquot of islets
    i. Verify accuracy of dithizone staining by immunofluorescence with DAPI, anti-insulin, and anti-glucagon antibodies
3) Stain purified islets with fluorescein diacetate and propidium iodide and assess islet viability by fluorescence microscopy

**C. Islet culture and handling of shipment**

1) Incubate islets at 37ºC in CMRL 1066 medium with 10% fetal calf serum, and ship at room temperature
2) Upon receipt of sample, reculture at 37ºC in CMRL 1066 medium with 10% fetal calf serum, supplemented with 100 U/mL penicillin and 100 U/mL streptomycin, for three days before execution of experiments.