OXYGEN CONSUMPTION PROTOCOL

I. Required Equipment/ Buffers

1. Oxygen partial pressure was measured by a phosphorescence quenching method, using a new oxygen-sensitive phosphorescent porphyrin-dendrimer Oxyphor G3 (palladium-tetrabenzoporphyrin, encapsulated inside gen 2 poly-arylglycine (AG) dendrimer)
2. Perfusion Apparatus:
    - peristaltic pump
    - water bath (37 °C)
    - a gas exchanger (artificial lung: media flowed through the thin-walled silastic tubing loosely coiled in a glass jar that contained 20% O_2 and 5% CO_2 balanced with N_2)
3. Krebs buffer (pH 7.4) containing 114 mmol/l NaCl, 5 mmol/l KCl, 24 mmol/l $NaHCO_3$, 1 mmol/l $MgCl_2$ $6H_2O$, 2.2 mmol/l Ca^{2+}, 1 mM P_i, 10 mmol/l HEPES (pH 7.4), 1% of BSA (fraction V, fatty acid free; Sigma-Aldrich) with the oxygen-sensitive phosphorescent porphyrin-dendrimer Oxyphor G3
4. Glucose
5. Amino Acid Mixture[1]
6. FCCP
7. NaN_3

II. Procedure

1. Using a dissecting microscope, handpick 500-1000 intact islets
2. Place islets on a filter in a 200µl glass perfusion chamber (Millipore, Bedford, MA)
3. Perfusion set to a flow rate of 50-80µl/min
4. Start perfusion system:
    - Krebs Buffer: No Substrate: 30 min
    - Add 4mM AAM: 30 min
    - Add 3mM Glucose: 30 min
    - Add 13.7mM Glucose (Final 16.7mM Glucose): 30min
    - Add 1.5mM FCCP: 30min
    - Add NaN_3: 42min
5. Islets were recovered from glass chamber and frozen as an islet pellet for DNA and hormone content measurements for normalization

[1] Amino Acid Mixture (AAM) in mM: 0.44 alanine, 0.19 arginine, 0.038 aspartate, 0.094 citrulline, 0.12 glutamate, 0.30 glycine, 0.077 histidine, 0.094 isoleucine, 0.16 leucine, 0.37 lysine, 0.05 methionine, 0.70 ornithine, 0.08 phenylalanine, 0.35 proline, 0.57 serine, 0.27 threonine, 0.073 tryptophan, and 0.20 valine, 2 mM glutamine.