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Protocol for Long-Term Islet Culturing

1. **PURPOSE:**
   This protocol describes how to culture islets for long term purposes.

2. **MATERIALS REQUIRED:**
   1. PIM(S)® media (Prodo labs – Cat no. - PIM-S001GMP).
   2. PIM(R)® media (Prodo labs-Cat no. – PIM-R001GMP).
   3. PIM(ABS)® (Prodo labs – Cat no. - PIM-ABS001GMP).
   4. PIM(G)® (Prodo labs – Cat no. - PIM-G001GMP).
   5. 35x10mm petri dish (Fisher, Sterile (Cat no. – 08-757-100A)).
   6. T-150 non-tissue culture treated flasks (Corning* Non-Treated Culture Flasks, Polystyrene, Sterile, (Cat no. - 431465).

3. **PROCEDURE:**
   To begin, follow the **Recovering Islets After Shipping Protocol**. To avoid or minimize the chance of contamination, the appropriate steps below are to be performed in a laminar flow hood with good sterile technique.

- **ISLET CULTURE**
  Short term islet culture is done in a 37°C incubator, with 5% CO_2, following the Culturing Islets Protocol.
  1. The PIM (S)® media on the islets in short term culture needs to be changed every 3-4 days. This is done by following the **Protocol for Islet Media Change**.
  2. Also, each time the media is changed, take islet samples for assessing viability/purity and place them in a 35mm petri dish.
  3. Islets can be cultured up to 10 days using this method.

- **EXTENDED CULTURING OF ISLETS (AFTER 10 DAYS)**
  Extended culturing is done using PIM(R) in a 37°C incubator, with 5% CO_2

  1. On the 10th day following the isolation, the islets need a 50% media change to PIM(R)®. This is done following the **Protocol for Islet Media Change.**
  2. The flasks are then placed in the 37°C incubator, with 5% CO_2.
  3. After this, step 1 is repeated every 3-4 days, using PIM(R)®
  4. Islets can be cultured for at least 4 weeks using this method