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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)<sup>®</sup> media (Prodo labs – Cat no. - PIM-S001GMP).
2. PIM(R)<sup>®</sup> media (Prodo labs-Cat no. – PIM-R001GMP).
3. PIM(ABS)<sup>®</sup> (Prodo labs – Cat no. - PIM-ABS001GMP).
4. PIM(G)<sup>®</sup> (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<sub>2</sub>, following the Culturing Islets Protocol.**

1. The PIM (S)<sup>®</sup> 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<sub>2</sub>**

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