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## Protocol for Culturing Islets

### 1. PURPOSE:

This protocol describes how to culture islets for short and long term purposes.

### 2. MATERIALS REQUIRED:

1. PIM(S)<sup>TM</sup> media (Prodo labs – Cat no. - PIM-S001GMP).
2. PIM(ABS)<sup>TM</sup> (Prodo labs – Cat no. - PIM-ABS001GMP).
3. PIM(G)<sup>TM</sup> (Prodo labs – Cat no. - PIM-G001GMP).
4. 60mm petri dish (VWR® Petri Dishes, Contact Plate, Sterile (Cat no. - 25384-093)).
5. Parafilm (VWR Cat no. - 52858-032).
6. T-150 non-tissue culture treated flasks (Corning \* Non-Treated Culture Flasks, Polystyrene, Sterile, (Cat no. 431465 or equivalent)).

### 3. PROCEDURE:

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>.**

1. The media on the islets in short term culture needs to be changed every 2-3 days. This is done by following the **Protocol for Islet Media Change**.
2. Also, each time the media is changed, take samples for assessing viability/purity and place them in a 60mm petri dish.
3. Islets can be cultured up to 2 weeks using this method.

#### • COLD STORAGE OF ISLETS

**Long term islet storage is done in a 8°C refrigerator/incubator .**

1. The flasks to be prepared for long term culture are taken from the incubator and their caps are sealed well with parafilm, to ensure no loss of CO<sub>2</sub> from the flask, and placed in the 8°C refrigerator/incubator .
2. Islets need to get their media changed every 7<sup>th</sup> day and allowed to resume to their normal metabolism overnight. This is done following the **Protocol for Islet Media Change**. However, all the media used will be cold.
3. In the first step of the **Protocol for Islet Media Change**, instead of warming up PIM(S) complete media, we cool it in a 2-8°C refrigerator prior to use.
4. The rest of the steps are the same as in the **Protocol for Islet Media Change**.
5. The flasks are then placed in the 37°C incubator, with 5% CO<sub>2</sub> overnight.
6. After this step 1 is repeated.
7. Islets can be cultured up to a month using this method.