Protocol for Culturing Islets

1. PURPOSE:

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

2. MATERIALS REQUIRED:

- TM0199-A
- TM0199-B
- 60 mm Petri dish - VWR® Petri Dishes, Contact Plate, Sterile (Cat no. 25384-093)
- Parafilm (VWR Cat. No. 52858-032)
- T-150 non-treated tissue culture flasks - Corning * Non-Treated Cell Culture Flasks, Polystyrene, Sterile (Cat no. 431465).

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.

4. ISLET CULTURE:

Short-term islet culture is done in a 37 °C incubator with 5% CO2.

1) The medium for 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 medium is changed, take samples for assessing viability/purity and place them in a 60 mm Petri dish.
3) Islets can be cultured up to around 2 weeks using this method.

EXTENDED CULTURING OF ISLETS (AFTER 10 DAYS)

Extended culturing is done using TM0199-B in a 37 °C incubator with 5% CO2.

1) On day 10, the islets need a 50% medium change to TM0199-B. This is done following the Protocol for Islet Media Change.
2) The flasks are then placed in the 37 °C incubator with 5% CO2.
3) After this, step 1 is repeated every 3-4 days, using TM0199-B.
4) Islets can be cultured for around 4 weeks using this method, depending on how well the procedure is performed.

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5. COLD STORAGE OF ISLETS

Long-term islet storage is done in an 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 CO2 from the flask, and placed in the 8 °C refrigerator/incubator.
2) Islets need to get their medium changed every 7th day and should be allowed to resume their normal metabolism overnight. This is done following the Protocol for Islet Media Change. However, all of the media used will be cold.
3) In the first step of the Protocol for Islet Media Change, instead of warming up TM0199-B complete medium, 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% CO2 overnight.
6) After this, step 1 is repeated.
7) Islets can be cultured up to approximately a month using this method, depending on how well the procedure is performed.

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