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PROTOCOL FOR DISTRIBUTING ISLETS IN TO MULTI WELL PLATES

1. **PURPOSE:** This protocol describes how to distribute islets evenly in to multi well plates

2. **RESPONSIBILITY:**

   It is the responsibility of the laboratory personnel who are responsible for the task to understand and follow this procedure.

3. **MATERIALS and EQUIPMENT:**

   3.1 Tissue culture flasks containing islets.
   3.2 Multi well plate of choice
   3.3 Islet culture media of choice
   3.4 50ml centrifuge tubes
   3.5 2ml Pipettes
   3.6 5ml Pipettes
   3.7 25 ml Pipettes
   3.8 50ml conical rack

4. **DEFINITIONS:**

   4.1 IEQ-Islet equivalence

5. **PROCEDURE:**

   NOTE: Islets can be distributed evenly in to 6well, 12 well or 24 well plates. Depending on the type of plate and the amount islets that are distributed, the volume will vary. An example is given as if the culture flask contains 10,000IEQ islets in 40ml culture media, and a 300IEQ islet each (which is 3600IEQ for a total of 12 wells) need to be distributed in to a 12 well plate.

   5.1 Work within a biological safety hood.
   5.2 Take the flask containing islets and take out the number of islets needed.
   This can be done by
   5.2.1 Calculate the volume that will contain 3600IEQ which is 14.4ml.
   5.2.1 Transfer the islets containing media in to a 50ml conical using a 25ml pipette.
   5.2.2 Mix the islets in the conical by drawing 25ml of the media with islets with a 25ml pipette from the bottom of the conical, and pipette it back slowly at the top of the media repeating it 5 times to get a good distribution.
   4.2.3 After mixing, go to the middle of the media and draw out 14.4ml (Which will contain 3600IEQ) in to a new 50ml conical.

5.2 Calculate the volume of media that will have 300IEQ which is 1.2ml.
5.3 Using a 5ml pipette mix the islets the same way described in step 4.2.2.
5.4 After mixing, go to the middle of the media and draw 1.2ml and distribute in to the 12 well plate.
5.5 Repeat step 4.4-4.5 until all the wells are filled with islets.