

Bestämning av antalet ö-ekvivalenter Estimation of total islet equivalents 710924

Estimation of total islet equivalents

Abbreviations and Codes

| | |
|-----|----------------------|
| IEQ | Islet Equivalents |
| rpm | rotations per minute |

Safety Rutines

All work with human material always carries a risk for transferring disease. See [Skyddsföreskrift laboratorielarbete AL4731](#).

Procedure

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Keep T-flasks cold. Work quickly. The islets should not be in density gradient solution longer than necessary!

1. Pool appropriate fractions into the centrifuge conicals. Pool material from max three T-flasks per conical, rinse the empty T-flasks and fill up to 250 ml with cold wash solution. Keep pure and unpure fractions separated but material from different COBE runs performed at the same time can be pooled together. Additional COBE runs are handled separate.
2. Centrifuge in the cold centrifuge for 3 min, 1000 rpm and 4°C.
3. Make sure that all material is in the pellet. If this is not the case, take the material left in the supernatant via a 25 ml pipette to a new conical and centrifuge again with the same settings.
4. Perform steps 1-3 with the remaining fractions.
5. Aspirate the supernatants (via vacuum suction) from packed pellets and avoid spreading the islets up the side of the flasks. Pool similar quality pellets (keep separate pure and unpure fractions) through careful resuspension and pool the fractions using a pipette or pouring into a 250 ml conical. Wash the empty conicals 2 times with about 25 ml of wash solution and transfer it to the pool. Check that the pellets have resuspended properly and add about 200 ml wash solution into the conical.
6. Centrifuge the conicals in the cold centrifuge for 1 min at 1000 rpm (225 g) and 4°C. If there is material left in the supernatant, transfer it to a new conical by using a serological pipette and centrifuge it at the same settings as before.
7. Estimate the pellet volume and enter it into the NICS database.

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8. Aspirate the supernatant and resuspend it in about 50 ml culture medium per 150 µl tissue. The culture medium should be complete with about 10% compatible human serum.
9. Mix the solution in the conical so the tissue is homogenous throughout the conical and take about 100-200 µl sample with a 2 ml pipette. The resuspended solution is taken care of according to the instruction "[Odling av öar. Islet culture AL5203](#)". This should happen as quickly as possible, but if not performed in a reasonable amount of time keep the islets cold.
10. Place the sample in a Petri dish and add about 4 drops of dithizone working solution.
11. Wait about 5 minutes until the dithizone has stained the islets red.
12. Estimate the sample purity as a percentage of stained tissue compared to the total amount of tissue (red colored islets and the yellow-brown exocrine material). Enter the purity into the NICS database (there is where calculation of total islet equivalents is made according to the following formula, see below).
13. Take pictures of the islets according to "[Fotografering av öar med Leica DFC490. Islet photography with Leica DFC490](#)"

Calculations and Protocol

The total islet equivalents (IE) calculated according to the following formula:

$$\text{IEQ} = \frac{\text{percent purity} \times \text{the volume of the pellet in } \mu\text{l} \times 1000}{230}$$

Protocol

All information is put into the NICS system.

Archiving

Protocol information is archived at least 10 years.

Equipment

Apparatus

| | |
|----------------------------|------------|
| Biological Safety Cabinets | MTA 075971 |
| Cold Centrifuge | MTA 074355 |
| Inverted Microscope | MTA 33898 |
| Vacuum Pump | MTA 215897 |

Material

| | |
|------------------------|--------|
| Conical, Corning 250ml | 746174 |
|------------------------|--------|

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| | |
|---|--------|
| Flasks, Corning black cap, 25cm ² (T-flasks) | 746180 |
| Pipette, 25 ml | 746195 |
| Pipette, 10 ml | 746065 |
| Pipet 2 ml | 746063 |
| Petri dish, Sterilin 50 mm | 746201 |
| Pipett-boy | 746253 |
| 5x8mm tubing for vacuum | 746218 |

Reagents/Additives

Reagents

| | |
|-----------------------------|--------|
| Wash solution | 767600 |
| Dithizone solution | 767596 |
| Culture medium | 767594 |
| Human serum, ABO compatible | 758220 |

Introduction

Estimation of total islet equivalents (IEQ, Islet Equivalents) from an islet isolation comes from the purity and the volume pancreas material remaining from a digested pancreas. The purity in this case concerns the part of the isolated pancreas material that is made of islets of Langerhans.

References

Related documents

[Fotografering av öar med Leica DFC490. Islet photography with Leica DFC490. AL5475](#)

[Odling av öar. Islet culture AL5203](#)

[Skyddsföreskrift laboratorielarbete AL4731](#)