Akademiska laboratoriet
Klinisk immunologi och transfusionsmedicin
Utförandebeskrivning

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PANCREAS DIGESTION

Safety routines, environmental, and work risks

Dithizone is classified as a poison.

All work with human material carries the risk of transmitting infectious diseases. See Skyddsföreskrift KITM and Hygienregler för Akademiska Sjukhuset AL9112.

Procedure

Preparations

Before the expected time to stop the digestion, prepare for the harvest:

- Clamp the serum tubing and weld it together with the serum bag. Serum tubing is connected to the first T-connector with luer connection located on the efferent tubing coming out from the chamber. Place the tubing in the pumphead of the Watson-Marlow 120U pump and hang the serum bag from the rod holding bag warmer in place.
- Make sure that Ringer's acetate for harvest "skördeflaskor" have been prepared according to "Lösningsprotokoll och odlingsmedium".
- Ensure that there is pulmozyme in the hood where the harvest conicals will be processed.
- Keep the centrifuge cold by centrifuging at 4° C just before harvesting.
- Place a cold insulated conical rack in the collection hood.
- Put a bag with UW for harvest and a refreezable ice pack into the pressure cuff. Hang this up inside the collection hood.

Procedure

The total time for the digestion varies depending on different collagenase batches and pancreases but should not exceed 30 minutes.

1. Add some water into the temperature port on the chamber and fasten the electronic temperature sensor onto the port.
2. Adjust the temperature in the chamber to obtain 37,5°C as soon as possible. Later on, adjust the setting on the bag heater during the digestion to maintain 37,5° C (*according to* Påsvärmare Biegler Protherm II. Blood bag heater Biegler Protherm II AL5224*)*.
3. Start shaking using 1-2 on the dial controlling speed for shaking. Increase the frequency by turning the dial to 6-7 for short intervals. Samples are taken from the 3-way connector with a syringe, see picture in instruction Montering av digestionssystemet. Assembling the digestion system AL5190. Collect about 3-5 ml per sample into

a petri dish. Stain each sample with dithizone (wait about 30 seconds before evaluation).

4. When there is a substantial increase in amount of material flow, change the shaking frequency to 6-7 continuously.
5. Determine the size of the islets and if they are free from the exocrine tissue. The size of the exocrine tissue should also be assessed. To help in deciding when to stop the digestion the following points can be used:
   - More than 50% of the islets need to be free from the exocrine tissue.
   - Exocrine tissue should have the same size as the biggest islets.
   - The total volume of material in a 3-5 ml sample should be about 20 µl (the material then has a diameter of 7-8 mm when swirled in the petri dish).
6. Take the cooling block for conical flasks out from the freezer and add 70 % ethanol (just enough so that when you put the conical in the cooling block, it almost pours out from the cooling block).
7. Put the cooling blocks for conical bottles on top of a sterile cloth in each hood.
8. Place the conicals into the cold block and collect the harvest into them.
9. Check the temperature, the time, and the amount and the size of the fragments in the tubing. Take the chamber off the shaking device and shake vigorously by hand intermittently and increase or decrease the temperature if needed. The digestion procedure varies in time and manner depending on the individual pancreas.
10. Take out a warm Ringer's acetate bottle for harvest completed with 30 ml "Skördtillsats".
11. When the digestion stops, the harvest will begin. This time will be marked in the NICS database. To make the harvesting possible the digestion system needs to be adjusted.
12. Stop the pump and take down the ringer acetate bottle. Clamp tube C. Draw out tube C together with the spike and remove the spike. Connect tube C to a sterile metal collection port, which is placed on a conical in the cooling block.
13. Remove the clamp on tube C, hang up the Ringer's acetate bottle, and start the pump.
14. Remove the clamp on the serum tubing and start the serum pump .
15. Before the Ringer's acetate bottle is emptied, stop the pump immediately or do following step quickly. Avoid pumping in the fat.
16. Remove tube D with the spike from the emptied Ringer's acetate bottle and move it to the Ringer's acetate bottle prepared according to step 8 above.
17. Hang the Ringer's acetate bottle up and start the pump.

18. Centrifuge the filled conicals in the cold centrifuge with the program set at 4° C, 1 min, 1000rpm. Write down the volume of the pellet after centrifugation.
19. Put the centrifuged conicals in the cooling block in the harvest hood and remove the supernatant with the vacuum apparatus.
20. Suspend each pellet in few ml of cold UW for harvest.
21. Pool the suspended pellets in cold conical and add 0.5 ml pulmozyme to the pooling conical. As soon as the pellet volume reaches above approximately 35 ml in the first conical, make a new pooling conical. Repeat this process for subsequent conicals.
22. The emptied 250 cc conicals are returned to the harvesting hood to be reused.
23. The following guidelines can be used during the harvest to avoid over digestion:
    - After switch to harvest shake the chamber for about 5 minutes, temperature should be about 37,5°C.
    - Then turn the chamber upside down and shake for 30 seconds, take a sample and turn the chamber right side up again.
    - Evaluate the sample, if there are a lot of embedded islets and big exocrine clumps continue harvest with the chamber right side up. Retake the sample at steady interval until the chamber can be turned upside down.
    - When the pellet volume rises to 2-3 ml temperature can be decreased to 37°C and when the pellet volumes further increase to about 5 ml accompanied by constant material flow the temperature can further be reduced to about 30°C. This is regardless if the chamber is turned upside down or not
24. Be observant to the level of Ringer's acetate left in the bottle. Turn off the pump when changing Ringer's acetate bottles. Turn the pump on as soon as the change has been completed. Alternatively change the bottle in about 3 seconds without turning off the pump.
25. Periodically check to see how the islets look using dithizone staining. Make sure that the temperature isn't too high and that the islets continually meet the criteria for the start of harvesting (see step 3 above). If the tissue fragments are too big, stop the shaking device and the pump and shake the chamber by hand 3-10 times. A sign to end the harvest is that the pellet volume has decreased and that about 60 minutes has passed on the timer (pancreas in the chamber).
26. End the harvesting by drawing out the spike from the last Ringer's acetate. Make sure the spike stays inside to hood.
27. Note the time harvesting ends, total number of used Ringer's acetate bottles, total pellet volume before centrifugation, and the weight of the tissue left in the chamber. All of this should go into the NICS database.

28. Centrifuge the pooled harvest conicals with the settings +4°C, 1 min, 1000 rpm. Remove the supernatant to another conical with a 50 ml serological pipet and centrifuge it with the same settings. Remove the supernatant with the vacuum and add the pellet from the supernatant conical to that of the original pooled conical. Enter the total volume of the centrifuged pellet volume into the NICS database.
29. The maximum pellet volume for each COBE separation is 45 ml. If the total pellet volume is above 45 ml, the pellet should be split into 2 (and if above 90 ml split into 3).
30. Resuspend the pellet and fill the conical with 100 ml 1x UW. Add 0.5 ml of Pulmozyme per COBE separation.
31. Prepare an incubation bag with small side bag by adding approximately 50 ml of wash solution to the small side bag and close the connection wetween the bags (for the wash step when after loading the islets on the COBE).
32. Transfer the cell suspension into the prepared incubation bag. Rinse the conical with approximately 50 ml 1xUW and add that to the bag. The total 1xUW volume should be 150 ml plus the pellet volume (max 195 ml) per incubation bag. Note the time in the NICS database.
33. Continue directly to purification step through gradient separation according to Gradientseparering med datorstyrda pumpar. Gradient separation with computer controlled pumps AL5477

Protocol

All information should be entered into the isolation and the database protocols.

Archiving

Protocol information is archived a minimum of 10 years.

Equipment

Apparatus

Biological Safety Cabinets	MTA 075957, 079370
Bag warmer, Biegler	MTA 072035
Shaking apparatus	MTA 072033
Digestion pump Watson Marlow 323	MTA 214073
Digital thermometer	MTA 073082, 074190
Inverted microscope	MTA 33898
Refrigerated centrifuge	MTA 074354
Vacuum pump	MTA 074200

Material

Metal collection adaptor	746171
Conical, Corning 250 ml	746174
Cooling block for cornical bottles	746304
Petri dish, Sterilin 50mm	746201
Pipet, 2 ml	746063
Pipet, 25 ml	746195
Pipetboy	746253
Pressure bag	746235

Reagents

Reagents

Dithizone working solution	767596
Ringer's Acetate with additives	767583
EtOH 70 %	758033
Human serum	758220
Pulmozyme	758297
1xUW for harvest and incubation	767597

Overview

The digestion of the pancreas will occur mechanically as well as enzymatically. It needs to be digested at 37° C to maximize enzyme activity. When the enzyme has been injected the pancreas is put into a chamber where 37° C buffer is circulating. In the chamber, which is shaken vigorously during the digestion, there are also metal/silicon nitride marbles that help to mechanically break down the pancreas.

References

Related documents

Montering av digestionssystemet. Assembling the digestion system AL5190

Gradientseparering med datorstyrda pumpar. Gradient separation with computer controlled pumps AL5477

Påsvärmare Biegler Protherm II. Blood bag heater Biegler Protherm II AL5224

Hygienregler för Akademiska Sjukhuset AL9112

Digerering av pankreas Pancreas digestion 710921