Application Note

Islet Isolation from Pigs with Collagenase NB 8 Broad Range and Neutral Protease NB

Equipment:

- Syringe
- Chopping scissors or scalpel
- Ricordi Chamber with marbles and mesh

Chemicals and Buffers:

- Collagenase NB 8 Broad Range (Cat. No. S1745602)
- Neutral Protease NB (Cat. No. S3030112)
- Pefabloc SC
- Dithizone
- Perfusion solution: Solution supplemented with Pefabloc SC (4 mM)

Procedure:

1. Calculate the required amount of Collagenase NB 8 Broad Range (4.4 PZ U/g pancreas). For preparing the Collagenase working solution weigh out the required amount of Collagenase NB 8 Broad Range and add 100 ml perfusion solution. If stock solutions were prepared before, dilute them accordingly with perfusion solution (4 °C).

2. Calculate the required amount of Neutral Protease NB: 0.5 – 0.7 DMC U/g pancreas (consider the neutral protease content in Collagenase NB 8 Broad Range). For preparing the Neutral Protease working solution weigh out the required amount of Neutral Protease NB and add 5 ml water (4 °C). If stock solutions were prepared before dilute them accordingly with water.

3. Shake both solutions gently on ice until dissolved and filter sterilize with a 0.22 µm filter. Place in refrigerator or on ice and use them within two hours after preparation.

4. Place the pancreas on a tray which is cooled on ice. Remove fat and membranes from the surface of the pancreas.

5. Cannulate the pancreatic duct, insert the cannula as far as possible into the head of the pancreas and close the secondary duct of the pancreas.

For information and samples please contact us:

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Ordering

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6. Mix the Collagenase NB 8 Broad Range solution with the Neutral Protease NB solution and add perfusion solution to a total volume of 180 ml. Keep the solution on ice and use it immediately.

7. Distend the pancreas manually with the total volume of the cold collagenase/neutral protease solution using a syringe.

8. Cut the perfused pancreas into several pieces.

9. Warm the perfusion solution to 37 °C.

10. Put the perfused pancreas pieces into the Ricordi chamber on top of the marbles and add the collagenase/neutral protease solution that leaked from the pancreas during perfusion and cutting.

11. Fill up the chamber with perfusion solution (37 °C) and place a mesh on top of the chamber to avoid blockage.

12. Reassemble the Ricordi chamber and let the solution circulate through chamber by a pump.

13. Adjust temperature to 37 °C as fast as possible and start shaking manually or automatically. Monitor changes of the pH value since it should not drop below 7.1.

14 Aspire samples (2 - 3 ml) every three to four minutes, stain with 1 – 2 ml dithizone and monitor the dissociation of the pancreatic tissue under a microscope.

15. When approximately 50 % free islets can be detected, stop the dissociation by diluting with cold perfusion solution and incubate for about 10 min.

16. Open the chamber and remove the residual large tissue fragments using a mesh.

17. Rinse with cold solution and fill the cell suspension into centrifugation tubes.

18. Centrifuge at 4 °C and 500 x g and discard the supernatant.

19. Wash the pellets with cold perfusion solution.

20. Proceed with purification of the isolated islets.

Ordering Information

Product	Cat. No.	Pack size
Collagenase NB 8 Broad Range	S1745601	250 mg
Collagenase NB 8 Broad Range	S1745602	1 g
Neutral Protease NB	S3030111	50 U
Neutral Protease NB	S3030112	100 U

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