Protocol for human islet isolation

Product Information

General
Collagenase NB 1 GMP and Neutral Protease NB GMP are used for dissociation of pancreatic tissues of different species (e.g. human, pig) for isolation of islets of Langerhans. The Collagenase NB 1 producing strain of Clostridium histolyticum has been carefully selected for producing a collagenase product that is non-toxic according to the requirements of the European Pharmacopoeial Test for Abnormal Toxicity ("General Safety Test"). Collagenase NB 1 and Neutral Protease NB are highly purified by a series of chromatographic steps. Collagenase NB 1 contains collagenases class I and II and an extremely low concentration of endotoxin.

Specification
Collagenase NB 1 GMP Grade:
- Collagenase activity: \( \geq 3.0 \text{ U/mg} \) (PZ acc. to Wünsch)
- Neutral protease activity: \( \leq 0.05 \text{ U/mg} \) (DMC)
- Trypsin-like activities: \( \leq 0.5 \text{ U/mg} \) (BAEE)
- Endotoxin: \( \leq 10 \text{ EU/mg} \)

Neutral Protease NB GMP Grade:
- Neutral protease activity: \( \geq 0.5 \text{ U/mg} \) (DMC)

Application
Collagenase NB 1 and Neutral Protease NB are designed for dissociation of pancreatic tissues of different species (e.g. human, pig) for isolation of islets of Langerhans, which are intended for human transplantation. The enzymes are not intended for use in humans. Responsibility for clinical use and the methods to isolate, purify and transplant islets lies solely with the providing physician/researcher and the responsible ethics commission.

Storage conditions
Both enzymes are provided separately as lyophilized powders and should be stored in a dry state:
- Collagenase NB 1 at +2 to +8 °C
- Neutral Protease NB at +2 to +8 °C

Instruction for use¹

General
Below, a protocol to achieve optimal isolation results with Collagenase NB 1 and Neutral Protease NB is described.

Chemicals/ Solutions
- Hank’s Balanced Salt Solution (HBSS)
- UW Solution
- Pefabloc SC or AEBSF-HCL, SERVA Electrophoresis (Cat. No. 31682.02 or 12745.03)
- HEPES, SERVA Electrophoresis (Cat. No. 25245.02)
- NaHCO₃, SERVA Electrophoresis (Cat. No. 30180.02)
- CaCl₂, SERVA Electrophoresis (Cat. No. 15585.02)
- Collagenase NB 1, SERVA Electrophoresis (Cat. No. 17455.03 or 17452.01 (GMP Grade))
- Neutral Protease NB, SERVA Electrophoresis (Cat. No. 30301.12 or 30303.01 (GMP Grade))
- Dithizone (DTZ)
- Human albumin

Buffer preparation
1. Perfusion Solution (PS)
   - HBSS + 0.35 g/L NaHCO₃ + 25 mM HEPES + 3.1 mM CaCl₂ (ready to use)

2. AEBSF-HCL - Stock Solution (40 mM)
   - 500 mg AEBSF-HCL
   - add 50 ml of Perfusion Solution
   - prepare 2 ml aliquots in syringes
   - store at -20 °C
   - filter through a 0.45 µm-filter unit after thawing

3. Neutral Protease NB Stock Solution (20 DMC-U/ml) (NPS)
   - 100 DMC-U Neutral Protease NB
   - add 5 ml aqua bidest., dissolve completely
   - filter through a 0.45 µm-filter unit
   - keep on ice
   - do not store the reconstituted enzyme for more than 2 hours

4. Collagenase Stock Solution (40 PZ-U/ml)(CSS)
- 2000 PZ-U Collagenase NB 1
- add 50 ml Perfusion Solution, agitate at 4 °C for 15 to 30 min until complete reconstitution
- filter through a 0.45 µm-filter unit
- keep on ice
- do not store the reconstituted enzyme for more than 2 hours

5. Collagenase Working Solution (CWS)
- determine the approximate pancreas weight (g)
- calculate the required volume (ml) of CWS (A): pancreas (g) * 2 ml/g
- calculate the required volume (ml) of NPS (B): (pancreas (g) * (0.5 - 0.8 DMC-U/g)) / 20 DMC-U/ml
- calculate the required volume (ml) of CSS (C): (pancreas (g) * 20 PZ-U/g) / 40 PZ-U/ml
- calculate the required volume (ml) of the AEBSF-stock (D): (pancreas (g) * 2 ml/g * 0.4 mM) / 40 mM
- calculate the required volume (ml) of PS (E): A - (B + C + D)
- combine B, C, D and E to obtain the final volume of CWS (A)
- keep on ice until use
- prepare CWS directly before use
- discard the remaining stock solutions

Alternatively, one complete Collagenase NB 1 vial can be used for dissociation of one pancreas:
- determine the approximate pancreas weight (g)
- calculate the required volume (ml) of CWS (A): pancreas (g) * 2 ml/g
- calculate the required volume (ml) of NPS (B): (pancreas (g) * (0.5 - 0.8 DMC-U/g)) / 20 DMC-U/ml
- calculate the required volume (ml) of the AEBSF-stock (C): (pancreas (g) * 2 ml/g * 0.4 mM) / 40 mM
- combine B, C and the complete volume of CSS
- add PS to obtain the final volume of CWS (A)
- keep on ice until use
- prepare CWS directly before use
- discard the remaining stock solutions

Pancreas dissection
1. Put the pancreas on an ice-cooled tray.
2. Resect carefully the attached part of the duodenum without damaging the pancreatic head or the gut.
3. Dissect roughly peripancreatic fat, lymphnodes and vessels from the resected pancreas without damaging the fibrous pancreatic capsule.
4. Separate the pancreatic head from the pancreatic corpus with a scalpel leaving enough tissue at the head to ligate a catheder in place after cannulation of the duct.
5. Repeat cannulation for the pancreatic tail.
6. Determine the net weight of pancreas.
7. Distend the pancreas by intraductal perfusion of the complete amount of Collagenase Working Solution (CWS) utilizing a perfusor syringe or a peristaltic pump connected to a pressure gauge.
8. Keep pressure at 80 mm Hg during first perfusion. Pour leaked solution through a mesh and repeat perfusion two times at a pressure of approximately 180 mm Hg.
9. Check for leaks during perfusion and seal them with appropriate clamps or tissue glue.
10. Remove remaining non-parenchymatic and non-distended tissue from the gland.

Pancreas dissociation
1. Assemble digestion chamber, temperature probe, tubing system, heating device and peristaltic pump.
2. Load the distended pancreas, leaked Collagenase Working Solution and 6 to 9 marbles into the digestion chamber.
3. Position the 500 µm-pore sized steel mesh inside the chamber and fix the chamber top.
4. Activate peristaltic pump with a speed of 150 ml/min and fill the digestion system with the appropriate amount of warm HBSS (24°C).
5. Recirculate the solution continuously with a flow rate of 150 ml/min.
6. Adjust the heating device to reach a maximum system temperature of 37 °C within 5 min.
7. Start agitation of the digestion chamber performed either manually or automatically.
8. Aspirate 2 - 3 ml samples every three to four minutes, stain with 1 - 2 ml of Dithizone and monitor dissociation under a microscope.
9. Stop recirculation if exocrine tissue is finely dispersed and significant numbers (10 - 20) of cleaved or marginally exocrine-attached islets appear in the biopsies.
10. Select the appropriate temperature according to the characteristics of the biopsies.
11. Collect the digested tissue in cooled 250 ml-conical tubes containing 20 ml of human serum albumin.
12. Centrifuge filled tubes for 1 min with 100 - 150 x g at 4 °C and discard the supernatant.
13. Combine pelleted tissue in cold UW Solution. Suspend 25 ml of tissue at maximum in 250 ml UW Solution.
14. Repeat washing of digested tissue in fresh UW Solution and store it for 45 - 60 min at 4 °C until continuous density gradient purification is performed.
15. Proceed with purification.