

**Collagenase NB 8 Broad Range**

**Protocol for isolation of pancreatic islets from pig**

**Product Information**

<b>General</b>	Collagenase NB 8 is used for dissociation of tissues of different species (e.g. human, rat, pig) for isolation of various cell types. In some cases Neutral Protease NB has to be added to the digestion. The Collagenase NB 8 producing strain of <i>Clostridium histolyticum</i> has been carefully selected for producing high amounts of collagenase and a balanced ratio of secondary proteases. Collagenase NB 8 is chromatographically purified and therefore contains reduced levels of secondary proteases and endotoxin.
<b>Specification</b>	<p><b>Collagenase NB 8:</b></p> <p>Collagenase activity                    ≥ 0.9 U/mg (PZ acc. to Wunsch)</p> <p>Neutral protease activity            ≤ 0.2 U/mg (DMC)</p> <p>Trypsin-like activities                ≤ 0.5 U/mg (BAEE)</p> <p>Endotoxin                                ≤ 100 EU/mg</p> <p><b>Neutral Protease NB:</b></p> <p>Neutral protease activity            ≥ 0.1 U/mg (DMC)</p>
<b>Application</b>	Collagenase NB 8 is used for dissociation of tissues of different species (e.g. human, rat, pig) for isolation of various cell types which are used for research purposes. The enzyme is not intended for use in humans.
<b>Storage conditions</b>	+2 to +8 °C

**Instruction for use**

<b>Chemicals/ Solutions</b>	<ul style="list-style-type: none"> <li>• University of Wisconsin Solution (UW Solution)</li> <li>• Pefabloc SC, SERVA Electrophoresis (Cat. No. 31682)</li> <li>• Collagenase NB 8, SERVA Electrophoresis (Cat. No. 17456)</li> <li>• Neutral Protease NB, SERVA Electrophoresis (Cat. No. 30301)</li> <li>• Dithizone (DTZ)</li> </ul>
<b>Buffer preparation</b>	<p><b>1. Perfusion Solution (PS)</b> UW Solution supplemented with Pefabloc SC (final concentration 4 mM)</p> <p><b>2. Collagenase NB 8 + Neutral Protease NB Working Solution (CNPW)</b></p> <ul style="list-style-type: none"> <li>• <b>Reconstitution of the enzymes</b> <ul style="list-style-type: none"> <li>• Calculate the PZ-activity you need: 4.4 PZ-U/g pancreas</li> <li>• Reconstitute the respective amount of Collagenase NB 8 in 100 ml PS by agitating at 4 °C for 5 min</li> <li>• Calculate the DMC-activity you need: 0.5 - 0.7 PZ-U/g pancreas (consider neutral protease content in Collagenase NB 8)</li> <li>• Reconstitute Neutral Protease NB in 5 ml water in the vial at 4 °C</li> </ul> </li> </ul> <p>Keep the reconstituted enzymes on ice Do not store the reconstituted enzymes for more than 2 hours</p> <ul style="list-style-type: none"> <li>• Add the respective amount of Neutral Protease NB to the reconstituted Collagenase NB 8 solution directly before use</li> <li>• Add with PS to a final volume of 180 ml</li> </ul> <p>Discard remaining Neutral Protease NB solution Sterile filtration if desired (use sterile filters with low protein binding properties)</p> <p>Keep CNPW on ice Prepare CNPW directly before use</p>

<b>Pancreas dissection</b>	<ol style="list-style-type: none"> <li>1. Put pancreas on a tray which is cooled on ice</li> <li>2. Remove fat and membranes from the surface of the pancreas</li> <li>3. Cannulate the pancreatic duct, insert cannula as far as possible into the head of the pancreas</li> <li>4. Close secondary duct of the pancreas</li> <li>5. Perfuse the pancreas: Distend manually with the whole volume of cold CNPW using a syringe</li> </ol>
<b>Pancreas dissociation</b>	<ol style="list-style-type: none"> <li>1. Cut the perfused pancreas into several pieces</li> <li>2. Prewarm PS at 37 °C</li> <li>3. Place perfused pancreas pieces and CNPW into the Ricordi chamber on top of the marbles</li> <li>4. Fill up the system with prewarmed PS</li> <li>5. Place mesh on top of the chamber to avoid blockage</li> <li>6. Reassemble chamber</li> <li>7. Circulate the solution with pump through chamber</li> <li>8. Adjust temperature to 37 °C as fast as possible</li> <li>9. Start shaking manually or automatically</li> <li>10. Monitor pH course (pH should not drop below 7.1)</li> <li>11. Aspire 2 - 3 ml samples every three to four minutes, stain with 1 – 2 ml Dithizone and monitor dissociation under a microscope</li> <li>12. When about 50 % free islets appear stop dissociation by dilution with cold PS and incubate for approx. 10 min</li> <li>13. Open the chamber and remove the residual large tissue fragments using a mesh</li> <li>14. Rinse with cold solution</li> <li>15. Fill the cell suspension into centrifugation tubes</li> <li>16. Centrifuge at 500 x g</li> <li>17. Discard the supernatant</li> <li>18. Wash the pellets with cold PS</li> <li>19. Proceed with purification</li> </ol>