

## Protocol for isolation of human adipocytes (adult)

### Product Information

<b>General</b>	<p>Collagenase NB 6 GMP Grade is designed for dissociation of human tissue for isolation of different cell types which are intended for transplantation in humans.</p> <p>The aseptic production process complies with the requirements of Annex 18 to the EU-Guide to Good Manufacturing Practices "GMP for active pharmaceutical ingredients". It therefore guarantees for reproducibility and traceability of batches. The documentation comprises all production, control and distribution records. Also the requirements of the European Pharmacopoeia are observed, as far as applicable.</p> <p>Documentation concerning Collagenase (e.g. viral and TSE safety, stability data) is available for approval of the cell isolation procedure by the authorities.</p> <p>The Collagenase NB 6 producing strain of <i>Clostridium histolyticum</i> 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"). A balanced ratio of collagenase and other proteases guarantees for high yields of viable cells.</p> <p>For preliminary tests Collagenase NB 4 which is enzymatically equivalent to Collagenase NB 6 but not produced under GMP conditions can be used as a low cost alternative.</p>								
<b>Specification</b>	<table> <tr> <td>Collagenase activity</td> <td>≥ 0.1 U/mg (PZ acc. to Wunsch)</td> </tr> <tr> <td>Sterility (Ph. Eur.)</td> <td>Must comply</td> </tr> <tr> <td><i>Clostridia</i></td> <td>Must comply</td> </tr> <tr> <td>Abnormal Toxicity</td> <td>Must comply</td> </tr> </table>	Collagenase activity	≥ 0.1 U/mg (PZ acc. to Wunsch)	Sterility (Ph. Eur.)	Must comply	<i>Clostridia</i>	Must comply	Abnormal Toxicity	Must comply
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<b>Application</b>	<p>Collagenase NB 6 GMP Grade is exclusively designed for dissociation of different human tissues to isolate cells for transplantation into humans.</p> <p>The enzymes are not intended for use in humans.</p> <p>Responsibility for clinical use and the methods to isolate, purify and transplant cells lies solely with the providing physician/researcher.</p>								
<b>Storage conditions</b>	<p>Collagenase NB 6 GMP Grade is provided as a lyophilized powder and should be stored <b>in a dry state</b> at +2 to +8 °C (Stability data acc. to ICH guidelines for 5 years).</p> <p>The dissolved enzyme (in Tris buffer, pH 7.1) is stable at below -20 °C (Stability data acc. to ICH guidelines for 1 year available).</p>								

### Instruction for use

<b>General</b>	<p>Below, a protocol is described to achieve optimal isolation results for human adipocytes (adult) with Collagenase NB 6 GMP Grade.</p>
<b>Chemicals/ Solutions</b>	<ul style="list-style-type: none"> <li>• PBS</li> <li>• Collagenase NB 6, SERVA Electrophoresis (Cat. No. 17458)</li> <li>• DMEM/F12</li> <li>• FBS</li> <li>• Penicillin-Streptomycin</li> <li>• Amphotericin B; SERVA Electrophoresis (Cat. No. 47982)</li> <li>• Hoechst 33258 dye</li> </ul>
<b>Buffer preparation</b>	<p>Add to DMEM/F12:</p> <ul style="list-style-type: none"> <li>- FBS (final concentration 10 %)</li> <li>- Penicillin-Streptomycin (final concentration 100 U/ml)</li> <li>- Amphotericin B (final concentration 2.5 ng/ml)</li> </ul>

<b>Tissue dissociation</b>	<ol style="list-style-type: none"> <li>1. Dissect vessels and visible connective tissue from the adipose tissue, cut it in small pieces (3 to 4 mm) and fill into Falcon tubes.</li> <li>2. Prepare the Collagenase Working Solution (CWS): <ul style="list-style-type: none"> <li>• Determine the approximate volume of the tissue (A ml).</li> <li>• Calculate the Units of Collagenase NB 6 you need: <ul style="list-style-type: none"> <li>• Final volume: 2 x A ml.</li> <li>• Final concentration 0.3 U/ml (specific Collagenase activity see Certificate of Analysis).</li> </ul> </li> <li>• Dissolve Collagenase NB 6 in A ml of PBS.</li> <li>• Add CWS to adipose tissue into the Falcon tube.</li> </ul> </li> <li>3. Incubate at 37 °C for 30 to 60 min in a water bath with constant gentle agitation (Temperature should not vary more than +/-1 degree as adipocytes are very sensitive to temperature fluctuations).</li> <li>4. Stop digestion by adding 2 vol of DMEM/F12 (completed with FBS, Penicillin-Streptomycin, Amphotericin).</li> <li>5. Filter through a 120 µm mesh.</li> <li>6. Incubate the filtrate for 5 min at RT: Upper phase: adult adipocytes + oil, lower phase: stromal-vascular cells including pre-adipocytes.</li> <li>7. Centrifuge at 200 x g for 1 min using a 50 ml centrifugation tube.</li> <li>8. Collect adipocytes from the floating cell layer.</li> <li>9. Resuspend adipocytes in DMEM/F12 (completed with FBS, Penicillin-Streptomycin, Amphotericin).</li> <li>10. Centrifuge at 180 x g for 10 min.</li> <li>11. Discard supernatant.</li> <li>12. Repeat the washing step.</li> <li>13. Resuspend in DMEM/F12 (completed with FBS, Penicillin-Streptomycin, Amphotericin).</li> <li>14. Filter through a 250 µm nylon mesh.</li> <li>15. Filter through a 200 µm nylon mesh.</li> <li>16. Stain the cells with Hoechst 33258 dye (4 µg/ml).</li> <li>17. Count the cells using a fluorescence microscope.</li> </ol>
<b>Results</b>	Yield: 50.000-100.000 cells/25 cm <sup>2</sup> in DMEM/F12 (completed with FBS, Penicillin-Streptomycin, Amphotericin).