Collagenase A
From Clostridium histolyticum

**Collagenase A, EC 3.4.24.3 Lyophilizate**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>10 103 578 001</th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td>10 103 586 001</td>
<td>500 mg</td>
</tr>
<tr>
<td>Cat. No.</td>
<td>11 088 793 001</td>
<td>2.5 g</td>
</tr>
</tbody>
</table>

- **Cat. No. 11 088 793 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - From Clostridium histolyticum
  - 100 mg

- **Cat. No. 11 088 807 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 100 mg

- **Cat. No. 11 088 815 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 500 mg

- **Cat. No. 11 088 831 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 2.5 g

### Specific activity

- >0.15 U/mg lyophilizate (collagenase activity):
- 1 U is the activity which liberates in 1 min at 25°C 1 µmol 4-phenylazobenzyl-oxycarbonyl-L-prolyl-L-leucine from 4-phenyl-azobenzyl-oxycarbonyl-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine (substrate according to Wünsch) under assay conditions (4).

### Additional enzyme activities

- The preparations contain other enzyme activities, from which the following are routinely measured for each lot:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostripain</td>
<td>1 U catalyzes the hydrolysis of 1 µmol N-Q-benzoyl-L-arginine ethylester (BAEE) per min at 25°C and pH 7.6 after activation with 1 mM calcium acetate and 2.5 mM dithiothreitol.</td>
</tr>
<tr>
<td>Tryptic activity</td>
<td>With BAEE as substrate: 1 U is that enzyme activity which hydrolyzes 1 µmol BAEE in 1 min at 25°C and pH 7.6.</td>
</tr>
<tr>
<td>Protease activity</td>
<td>1 U is that protease activity which is causing an absorption increase of 0.001 in 1 min at 25°C in the standard azocoll test.</td>
</tr>
</tbody>
</table>

Collagenase A, B and D contain different ratios of the various proteolytic activities.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>11 088 858 001</th>
<th>2.5 g</th>
</tr>
</thead>
</table>

**Cat. No. 11 088 858 001**
- Clostridiopeptidase A, EC 3.4.24.3
- From Clostridium histolyticum
- 2.5 g

**Cat. No. 11 088 866 001**
- Clostridiopeptidase A, EC 3.4.24.3
- From Clostridium histolyticum
- 500 mg

**Cat. No. 11 088 831 001**
- Clostridiopeptidase A, EC 3.4.24.3
- From Clostridium histolyticum
- 2.5 g

Collagenase A, B, C and D are prepared from C. histolyticum cultures by filtration, ammonium sulfate precipitation, dialysis and lyophilization.

**Lot-specific data**

- **Collagenase D**
  - Cat. No. 10 103 578 001
  - 500 mg
  - Lot No.: 70147525 / 31 Aug 2008
  - Activity: 2.5 U/mg lyo.
  - Proteasen (Azocoll): 74 U/mg lyo.
  - Trypsin (BAEE): 0.069 U/mg lyo.

Collagenase D 11 088 866 001 500 mg

Lot-specific data:

- **Collagenase D**
  - Cat. No. 11 088 807 001
  - 100 mg
  - Cat. No. 11 088 815 001
  - 500 mg
  - Cat. No. 11 088 831 001
  - 2.5 g

**Collagenase B**
From Clostridium histolyticum

**Collagenase B, EC 3.4.24.3 Lyophilizate**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>11 088 807 001</th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td>11 088 815 001</td>
<td>500 mg</td>
</tr>
<tr>
<td>Cat. No.</td>
<td>11 088 831 001</td>
<td>2.5 g</td>
</tr>
</tbody>
</table>

- **Cat. No. 11 088 807 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 100 mg

- **Cat. No. 11 088 815 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 500 mg

- **Cat. No. 11 088 831 001**
  - Clostridiopeptidase A, EC 3.4.24.3
  - Lyophilizate
  - 2.5 g

Inhibitors
- EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol.

**Note:** Collagenase is not inhibited by serum.

**Activators**
- Ca²⁺

**pH Optimum**
- 6.0–8.0

**Application**
- Collagenase from C. histolyticum is now widely used for the disaggregation of all kind of tissues (e.g. lung, heart, muscle, bone, adipose tissue, liver, kidney, cartilage, mammary gland, placenta, blood vessels, brain, all kind of tumors) and for the preparation of single cell suspensions for the establishment of primary cell culture systems.

Collagenase from Roche has been used to prepare cells from many types of tissue, e.g. hepatocytes, adipocytes, pancreatic islets, epithelial cells, muscle cells, endothelial cells etc. (5-19). However, suitability of each lot of the enzyme for disruption of a particular tissue should be determined empirically.

**Reconstitution**
In any balanced salt solution (e.g. HBSS) (see table 1).

**Tab. 1:** Composition of selected balanced salt solutions ¹

<table>
<thead>
<tr>
<th>Ringer²</th>
<th>Tyrode³,⁴</th>
<th>Gey⁵</th>
<th>Earle⁶</th>
<th>Puck⁷</th>
<th>Hanks⁸</th>
<th>Dulbecco (PBS)³,⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>9.00</td>
<td>8.00</td>
<td>7.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>KCl</td>
<td>0.42</td>
<td>0.20</td>
<td>0.37</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>CaCl ₂</td>
<td>0.25</td>
<td>0.20</td>
<td>0.17</td>
<td>0.20</td>
<td>0.125</td>
<td>0.14</td>
</tr>
<tr>
<td>MgCl ₂ × 6 H2O</td>
<td>0.10</td>
<td>0.21</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>MgSO ₄ × 7 H2O</td>
<td>0.07</td>
<td>0.10</td>
<td>0.154</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>NaH2PO4 × 12 H2O</td>
<td>0.30</td>
<td>0.39</td>
<td>0.12</td>
<td>2.31</td>
<td>2.31</td>
<td>2.31</td>
</tr>
<tr>
<td>NaHPO₄ × 2 H2O</td>
<td>0.08</td>
<td>0.125</td>
<td>0.08</td>
<td>0.125</td>
<td>0.08</td>
<td>0.125</td>
</tr>
<tr>
<td>NaClO₃</td>
<td>0.03</td>
<td>0.15</td>
<td>0.06</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>NACL₂</td>
<td>1.00</td>
<td>2.27</td>
<td>2.20</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.05</td>
<td>0.05</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>air</td>
<td>air</td>
<td>99% air/ 9% CO₂</td>
<td>air</td>
<td>air</td>
<td>99% air/ 9% CO₂</td>
</tr>
</tbody>
</table>

¹ Amounts are given as grams per liter of solution.
⁹ PBS, phosphate-buffered saline.
**Working concentration**
approx. 1 mg/ml (0.1%, w/v).

**Storage/Stability**
The lyophilizate is stable at 2–8°C, when stored dry and protected from light, until the expiration date printed on the label.
The reconstituted solution is stable at −15 to −25°C.

**Background information**
Bacterial collagenase, or more accurately clostridiopeptidase A, is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are found in high frequency in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue (1–3).

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix.

The collagenase most commonly used for tissue dissociation is a crude preparation from C. histolyticum containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases. Crude collagenase is apparently ideally suited for tissue dissociation since it contains the enzyme required to attack native collagen, in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracellular matrix of tissues.

Collagenase A, B and D are prepared from the extracellular culture filtrate of Clostridium histolyticum. These crude preparations contain collagenase and other proteolytic activities. This allows for selection of the preparation best suited for dissociation of a particular tissue.

Collagenase from Roche is assayed in Wünsch units (1 µmol of product formed per minute at 25°C with Wünsch substrate [4]).

Frequently, collagenase activities are given in Mandl units (1 µmol leucine liberated from collagen in 5 h at 37°C). Unfortunately, there is no consistent conversion factor between the two units of activity, since the Mandl unit depends, in part, on the concentration of contaminating proteases in the collagenase preparation, an indefinite variable. A purer collagenase preparation would actually give a lower specific activity in Mandl units than a crude preparation. Clostridium preparations typically give conversion factors of approx. 1:1800 (e.g. a particular lot of Clostridium collagenase contained approx. 0.15 Wünsch U/mg and 250 Mandl U/mg).

### 2. Procedures and required materials

#### General
Two types of procedures are commonly used. The first involves mincing tissue and incubating the pieces in a collagenase solution with mild agitation. Cells are gradually released from the tissue during the collagenase treatment. The second involves perfusing an organ with the collagenase solution. Cells are gradually released into the perfusate or the tissue is then dissociated by mild mechanical treatment.

#### Additional reagents and material required
- PBS, sterile
- Filter membrane, 0.22 µm
- Nylon mesh or gaze

#### Protocol
The following working instruction describes, as an example, a procedure for minced tissue:

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dissolve the non-sterile, lyophilized enzyme in a balanced salt solution and filter sterilize through a 0.22 µm filter membrane.</td>
</tr>
<tr>
<td>2</td>
<td>Wash the tissue in sterile PBS or another balanced salt solution.</td>
</tr>
<tr>
<td>3</td>
<td>Remove undesirable tissue like fat or necrotic material and cut the remaining tissue with a scalpel into 1-3 mm cubes.</td>
</tr>
<tr>
<td>4</td>
<td>Add collagenase solution (usually 0.1% to 0.25% [w/v]). <strong>Note:</strong> It is possible, but in most cases not necessary, to add other enzymes such as pro-nase*, hyaluronidase*, elastase* or trypsin* to the collagenase solution.</td>
</tr>
<tr>
<td>5</td>
<td>Incubate at 37°C until disaggregation is complete.</td>
</tr>
<tr>
<td>6</td>
<td>Check for effective disaggregation. If the cell suspension becomes viscous due to DNA release from digested cells, add DNase I* to alleviate this problem. If necessary separate undisassociated fragments from single cells by collecting the supernatant after allowing the fragments to settle and add fresh enzyme solution to the tissue fragments. The cell suspension can be passed through a nylon mesh or gaze to remove any undigested fragments.</td>
</tr>
<tr>
<td>7</td>
<td>Centrifuge the supernatant(s) at 50–100 × g for about 3 min.</td>
</tr>
<tr>
<td>8</td>
<td>Resuspend the pellet in medium and seed as usual. <strong>Note:</strong> For organ perfusion procedures special products (collagenase H* for hepatocyte isolation, collagenase P* for pancreatic islet isolation) are available.</td>
</tr>
</tbody>
</table>
3. References


4. Ordering Guide

For further information please access our web-site address at:
http://www.roche-applied-science.com

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase H</td>
<td>100 mg</td>
<td>11 074 032 001</td>
</tr>
<tr>
<td></td>
<td>500 mg</td>
<td>11 074 059 001</td>
</tr>
<tr>
<td></td>
<td>2.5 g</td>
<td>11 087 789 001</td>
</tr>
<tr>
<td>Collagenase P</td>
<td>100 mg</td>
<td>11 213 857 001</td>
</tr>
<tr>
<td></td>
<td>500 mg</td>
<td>11 213 865 001</td>
</tr>
<tr>
<td></td>
<td>2.5 g</td>
<td>11 213 873 001</td>
</tr>
<tr>
<td>Collagenase/Dispase</td>
<td>100 mg</td>
<td>10 269 638 001</td>
</tr>
<tr>
<td></td>
<td>500 mg</td>
<td>11 097 113 001</td>
</tr>
<tr>
<td>Pronase</td>
<td>1 g</td>
<td>10 165 921 001</td>
</tr>
<tr>
<td></td>
<td>5 g</td>
<td>11 459 643 001</td>
</tr>
<tr>
<td>DNase I</td>
<td>100 mg sterile</td>
<td>11 284 932 001</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>100 mg</td>
<td>10 106 500 001</td>
</tr>
<tr>
<td>Elastase</td>
<td>10 mg</td>
<td>11 027 891 001</td>
</tr>
<tr>
<td></td>
<td>50 mg</td>
<td>11 027 905 001</td>
</tr>
<tr>
<td>Trypsin</td>
<td>500 mg</td>
<td>10 109 819 001</td>
</tr>
<tr>
<td></td>
<td>2 g</td>
<td>10 109 827 001</td>
</tr>
<tr>
<td>Trypsin Solution</td>
<td>100 ml sterile</td>
<td>10 210 234 001</td>
</tr>
<tr>
<td>Buffers in a Box, premixed PBS Buffer, 10×</td>
<td>4 l</td>
<td>11 666 789 001</td>
</tr>
</tbody>
</table>

* available from Roche Applied Science

Contact and Support
To ask questions, solve problems, suggest enhancements or report new applications, please visit our Online Technical Support Site at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed.

Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.