

For life science research only.  
Not for use in diagnostic procedures.



# Collagenase P

## from *Clostridium histolyticum*

 **Version: 23**

Content Version: December 2020

Clostridiopeptidase A

<b>Cat. No. 11 213 857 001</b>	100 mg
<b>Cat. No. 11 213 865 001</b>	500 mg <i>Not available in US</i>
<b>Cat. No. 11 249 002 001</b>	1 g
<b>Cat. No. 11 213 873 001</b>	2.5 g

**Store lyophilizate at +2 to +8°C.**

<b>1.</b>	<b>General Information .....</b>	<b>3</b>
1.1.	Contents .....	3
1.2.	Storage and Stability .....	3
	Storage Conditions (Product) .....	3
	Storage Conditions (Working Solution).....	3
	Reconstitution .....	3
1.3.	Additional Equipment and Reagent required .....	4
1.4.	Application .....	4
<b>2.</b>	<b>How to Use this Product .....</b>	<b>5</b>
2.1.	Before you Begin .....	5
	Safety Information .....	5
	Laboratory procedures .....	5
	Waste handling.....	5
	Working Solution.....	6
2.2.	Protocols .....	7
	Preparation of the pancreas .....	7
	Method I - Isolation of islets.....	7
	Method II - Isolation of islets .....	9
2.3.	Parameters .....	9
	Biological Activity .....	9
	EC-Number .....	9
	Specific Activity .....	9
	Unit Definition.....	10
	Working Concentration.....	10
<b>3.</b>	<b>Additional Information on this Product .....</b>	<b>10</b>
3.1.	Test Principle .....	10
	Preparation.....	10
3.2.	References .....	10
3.3.	Quality Control.....	10
<b>4.</b>	<b>Supplementary Information .....</b>	<b>11</b>
4.1.	Conventions.....	11
4.2.	Changes to previous version .....	11
4.3.	Ordering Information.....	11
4.4.	Trademarks.....	12
4.5.	License Disclaimer .....	12
4.6.	Regulatory Disclaimer.....	12
4.7.	Safety Data Sheet .....	12
4.8.	Contact and Support.....	12

# 1. General Information

## 1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Collagenase P	Lyophilized, nonsterile	11 213 857 001	1 vial, 100 mg
			11 213 865 001	1 vial, 500 mg
			11 249 002 001	1 vial, 1 g
			11 213 873 001	1 vial, 2.5 g

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Collagenase P	Store dry at +2 to +8°C.

### Storage Conditions (Working Solution)

Store reconstituted solution at –15 to –25°C.

### Reconstitution

Reconstitute Collagenase P in any balanced salt solution, such as HBSS (Hank's Balanced Salt Solution).

### 1.3. Additional Equipment and Reagent required

#### For preparation of the pancreas - Method I

**i** See section, **Working Solution** for additional information.

- HBSS (Hank's Balanced Salt Solution)
- HEPES
- Washing solution
- Ficoll
- Culture medium: Medium RPMI 1640 containing 10% FCS.
- Water bath
- Centrifuge
- Filter, mesh size 600 µm

#### For isolation of single cells from islets

**i** See section, **Working Solution** for additional information.

- HBSS (Ca<sup>2+</sup>/Mg<sup>2+</sup>-free)
- Trypsin solution
- Culture medium: Medium RPMI 1640 containing 10% FCS.
- BSA\*
- 18-gauge cannula
- 20-gauge needle
- Centrifuge
- CO<sub>2</sub> incubator

#### For preparation of the pancreas - Method II

**i** See section, **Working Solution** for additional information.

- Krebs-Ringer solution
- Water bath
- Stereo microscope

#### For additional isolation steps

- Ca<sup>2+</sup>-free Krebs-Ringer solution

### 1.4. Application

Collagenase P is an enzyme mixture used for the disaggregation of tissues and for the isolation of cells.

- Because of its high collagenase activity, the preparation is particularly suitable for the isolation of pancreatic islets. Each lot is tested for its suitability for the isolation of pancreatic islets from mouse or rat pancreas.
- This collagenase preparation is also suitable for the isolation of adipocytes from epididymal fat pads of rats.

**i** *Collagenase P is a non-sterile preparation of the culture supernatant of Clostridium histolyticum. It is neither designed nor intended for isolation of pancreatic islets for transplantation into humans and must not be used for such purposes.*

## 2. How to Use this Product

### 2.1. Before you Begin

#### Safety Information

##### Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

##### Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on [dialog.roche.com](http://dialog.roche.com), or upon request from the local Roche office.

## Working Solution

Reagent/Solution	Composition	For use in...
<b>Preparation of pancreas</b>		
HBSS	<ul style="list-style-type: none"> <li>▪ 8 g/l NaCl</li> <li>▪ 0.4 g/l KCl</li> <li>▪ 0.14 g/l CaCl<sub>2</sub></li> <li>▪ 0.1 g/l MgSO<sub>4</sub> × 7 H<sub>2</sub>O</li> <li>▪ 0.1 g/l MgCl<sub>2</sub> × 6 H<sub>2</sub>O</li> <li>▪ 0.06 g/l Na<sub>2</sub>HPO<sub>4</sub> × H<sub>2</sub>O</li> <li>▪ 0.06 g/l KH<sub>2</sub>PO<sub>4</sub></li> <li>▪ 1 g/l glucose</li> <li>▪ 0.02 g/l phenol red</li> <li>▪ 0.35 g/l NaHCO<sub>3</sub></li> </ul>	Preparation of Collagenase solution.
Collagenase solution	0.5 to 1.5 mg/ml Collagenase P in HBSS containing 10 mM HEPES. The following quantities are used from this solution: <ul style="list-style-type: none"> <li>▪ 12 ml for a mouse pancreas</li> <li>▪ 10 ml for a rat pancreas</li> </ul> <i>i</i> The optimal concentration of each collagenase P lot must be determined individually.	Step 7
<b>Method I - Isolation of islets</b>		
Washing solution	HBSS containing 10 mM HEPES and 5% FCS (fetal calf serum).	Step 3, 4, 8
Ficoll gradient	25% stock solution (Ficoll 400 in HBSS containing 10 mM HEPES) and 23%, 20%, and 11% dilutions.	Steps 6, 7
Culture medium	Medium RPMI 1640 containing 10% FCS.	Step 9
<b>Isolation of single cells from islets</b>		
HBSS (Ca <sup>2+</sup> /Mg <sup>2+</sup> -free)	<ul style="list-style-type: none"> <li>▪ 3 mM EGTA [Ethylenedis(oxyethylenenitrilo) tetraacetic acid]</li> <li>▪ 20 mg/ml BSA (bovine serum albumin)</li> <li>▪ 2.2 mg/ml glucose</li> </ul>	Step 2
Trypsin solution, 0.25%	2.5 mg/ml in PBS (phosphate buffered saline).	Step 3
Culture medium	Medium RPMI containing 10% FCS.	Steps 6, 7
<b>Method II - Isolation of islets</b>		
Krebs-Ringer solution	<ul style="list-style-type: none"> <li>▪ 115 mM NaCl</li> <li>▪ 4.7 mM KCl</li> <li>▪ 2.56 mM CaCl<sub>2</sub></li> <li>▪ 1.2 mM KH<sub>2</sub>PO<sub>4</sub></li> <li>▪ 1.2 mM MgSO<sub>4</sub> × 7 H<sub>2</sub>O</li> <li>▪ 20 mM NaHCO<sub>3</sub></li> <li>▪ 16 mM HEPES</li> </ul>	Step 1

## 2.2. Protocols

### Preparation of the pancreas

- 1 Sacrifice the animal and open the abdomen.

---

- 2 Uncover the bile duct by displacing the duodenum and the liver.

---

- 3 Clamp off the bile duct at its entrance to the duodenum.

---

- 4 Free the upper part of the bile duct by dissection to the liver.

---

- 5 Bleed the animal completely by heart puncture.

---

- 6 Inject Collagenase solution through the common bile duct into the pancreas.

---

- 7 Distend the pancreas with the Collagenase solution.

---

- 8 Remove the distended pancreas.

---

- 9 Transfer the pancreas to a 5 cm petri dish (mouse) or 10 cm petri dish (rat).

**⚠ Two possible working instructions for the isolation of pancreatic islets and islet cells are given below.**

### Method I - Isolation of islets

This method can be used to isolate islets and islet cells from mouse and rat pancreas. The islets are isolated by collagenase digestion and the isolation is accomplished using a Ficoll gradient. The islets can be subsequently dissociated into single cells.

- 1 Incubate for 45 to 50 minutes at +37°C in a water bath.

---

- 2 Pour the digested tissue into a 15 ml plastic tube; add approximately 10 ml Washing solution and suspend the tissue.  
**⚠ Perform the following steps at +2 to +8°C.**

---

- 3 Centrifuge for approximately 1 minute at 250 × g.  
– Discard the supernatant and wash twice with Washing solution.

---

- 4 After the second washing step, discard the supernatant, resuspend in 5 ml Washing solution, and pass through a filter (mesh size 600 µm).  
– Wash again with 5 ml of solution.  
**i For rat pancreas, distribute the digested tissue in several plastic tubes to avoid overloading.**

---

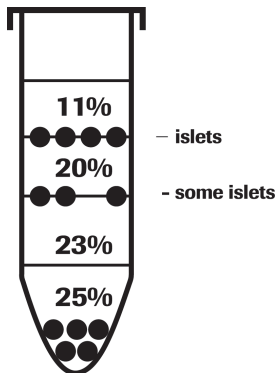
- 5 Centrifuge for approximately 1 minute at 250 × g.  
– Remove the supernatant completely.

---

- 6 Resuspend the pellet in 3 ml 25% Ficoll stock solution.

## 2. How to Use this Product

- 7 Load on top of the 3 ml 25% Ficoll suspension, a discontinuous density gradient of 23% (3 ml), 20% (2 ml), and 11% (2 ml) Ficoll solutions.
  - Centrifuge for approximately 10 minutes at  $800 \times g$ .
  - i* The islets are found at the interface of 11% and 20% after the centrifugation. Some islets are at the interface of 20% and 23% (**Fig. 1**).
- 8 Wash the islets obtained from the upper two interfaces three times in Washing solution (centrifuge for 1 minute at  $250 \times g$ ).
  - Resuspend pellet in 2 ml Washing solution.
- 9 Hand pick the islets and resuspend in culture medium.



**Fig. 1:** Concentration of islets.

### Isolation of single cells from islets

- 1 Centrifuge islets in culture medium for 5 minutes at  $250 \times g$ .
- 2 Discard supernatant and resuspend the pellet in 3 ml HBSS containing EGTA, BSA, and glucose.
- 3 Add 100  $\mu$ l trypsin solution.
  - Incubate for 10 minutes at  $+37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ .
- 4 Aspirate the islets 3 to 5 times through an 18-gauge cannula and transfer the cell suspension using a 20-gauge needle to another plastic tube containing 5 ml culture medium.
- 5 Centrifuge for 10 minutes at  $250 \times g$ .
- 6 Wash twice with culture medium.
- 7 Resuspend the pellet in culture medium.



## Method II - Isolation of islets

This method can also be used to isolate islets from mouse and rat pancreas.

- 1 Place the pancreas in a petri dish containing 5 ml (rat) or 2 ml (mouse) Krebs-Ringer solution.

---

- 2 Cut the pancreatic tissue into small pieces using scissors for approximately 1 minute.

---

- 3 Transfer the Krebs-Ringer solution with the minced tissue to an appropriate glass vial into which the necessary amount of Collagenase P has already been introduced. For example, 1 mg for a mouse pancreas or 4 to 5 mg for a rat pancreas, depending on the size of the organ.

---

- 4 Incubate the vial at +37°C in a water bath for a certain period of time, typically approximately 12 minutes, with rapid shaking (300 cycles/minute).

---

- 5 Shake the vial by hand, typically 10 to 60 seconds until the solution appears homogeneous.

---

- 6 Add 10 ml Krebs-Ringer solution and shake the solution again.

---

- 7 Allow the islets to settle for 3 minutes and remove the supernatant.  
– Fill the vial again with Krebs-Ringer solution and resuspend.

---

- 8 Transfer the suspension to a petri dish and pick the isolated pancreatic islets under a stereo microscope using an Eppendorf pipette.

---

### Additional isolation steps

Single cells can be isolated from these pancreatic islets in an additional step. If the islets are overdigested by the collagenase treatment, single cells can simply be isolated by vortexing in Ca<sup>2+</sup>-free Krebs-Ringer solution. If the islets are not overdigested, the cell isolation has to be performed using trypsin (see Method I).

## 2.3. Parameters

### Biological Activity

Tested for the isolation of pancreatic islets from mouse and rat pancreas.

### EC-Number

EC 3.4.24.3

### Specific Activity

>1.5 U/mg lyophilizate (collagenase activity)

The preparation contains other enzyme activities, from which the following are routinely measured for each lot:

Enzyme	Proteolytic activity
Clostripain activity	1 U catalyzes the hydrolysis of 1 µmol N-α-benzoyl-L-arginine ethyl ester (BAEE) per minute at +25°C and pH 7.6 after activation with 1 mM calcium acetate and 2.5 mM dithiothreitol.
Tryptic activity	With BAEE as substrate: 1 U is that enzyme activity which hydrolyzes 1 µmol BAEE in 1 minute at +25°C and pH 7.6.
Protease activity	1 U is that protease activity which is causing an absorption increase of 0.001 in 1 minute at +25°C in the standard azocoll test.

### 3. Additional Information on this Product

#### Unit Definition

1 U is the activity which liberates in 1 minute at +25°C, 1 µmol 4-phenyl-azobenzyl-oxycarbonyl-L-prolyl-L-leucine from 4-phenyl-azobenzyl-oxycarbonyl-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine (substrate according to Wunsch) under assay conditions (Wünsch E, Heidrich HG, 1963).

#### Working Concentration

**Isolation of pancreatic islets from mouse and rat pancreas:** 0.5 to 1.5 mg/ml

**Isolation of adipocytes:** approximately 2 mg/ml

**i** Refer to the Certificate of Analysis for the lot-specific concentration and additional lot-specific information.

## 3. Additional Information on this Product

### 3.1. Test Principle

#### Preparation

Collagenase P is prepared from the extracellular culture filtrate of a special *Clostridium histolyticum* strain.

**i** Collagenase P is a research biochemical and is not manufactured under the US Good Manufacturing Practices (GMP) regulations.

### 3.2. References

- Wünsch E, Heidrich HG. On the quantitative determination of collagenase. Hoppe Seylers Z Physiol Chem. 1963;333:149-151.

### 3.3. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

## 4. Supplementary Information


### 4.1. Conventions




To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

#### Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

   etc. Stages in a process that usually occur in the order listed.

   etc. Steps in a procedure that must be performed in the order listed.

\* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

### 4.2. Changes to previous version

Layout changes.

Editorial changes.

Update to include new safety Information to ensure handling according controlled conditions.

### 4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, <i>Not available in US</i>	10 735 086 001
	500 g, <i>Not available in US</i>	10 735 094 001
	1 kg, <i>Not available in US</i>	10 735 108 001

## 4. Supplementary Information

### 4.4. Trademarks

All product names and trademarks are the property of their respective owners.

### 4.5. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

### 4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

### 4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

### 4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

