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

Collagenase H
from *Clostridium histolyticum*

Version: 20
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Clostridiopeptidase A

Cat. No. 11 074 032 001	100 mg
Cat. No. 11 074 059 001	500 mg
Cat. No. 11 087 789 001	2.5 g

Store lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Collagenase H	Lyophilized, nonsterile	11 074 032 001	1 vial, 100 mg
			11 074 059 001	1 vial, 500 mg
			11 087 789 001	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 H	Store dry at +2 to +8°C.

Storage Conditions (Working Solution)

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

Reconstitution

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

1.3. Additional Equipment and Reagent required

For isolation of rat hepatocytes

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

- Sprague-Dawley rats
- Perfusion apparatus
- Sterile forceps
- Sterile beaker
- Culture Vessels
- 50 mm petri dish
- 74 mm filter or 4 sheets sterile gauze
- Perfusion medium, 20x conc.
- Culture medium
- FCS (fetal calf serum)
- Sodium pentobarbital, such as Nembutal
- Sodium heparin, such as Liquemin 25,000

For isolation of adipocytes

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

- Wistar rats
- Shaking water bath
- Plastic tubes, such as 50 ml centrifuge tubes
- 74 mm filters or 4 sheets sterile gauze
- Disintegration medium
- Buffer solution
- Culture medium

1.4. Application

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

- Collagenase H consists only of lots which have been tested for the suitability for the isolation of hepatocytes from rat liver by the collagenase perfusion method.
- This collagenase preparation is also suitable for the isolation of adipocytes from epididymal fat pads of rats and for the isolation of endothelial cells from various sources.

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, or upon request from the local Roche office.

Working Solution

Prepare working solutions according to the following table.

Reagent/Solution	Composition	For use in...
Isolation of rat hepatocytes		
Perfusion medium (20x stock solution)	▪ 138.0 g/l NaCl ▪ 8.9 g/l KCl ▪ 11.4 g/l Na_2HPO_4 ▪ 5.4 g/l NaH_2PO_4 ▪ 180.0 g/l glucose ▪ 6.0 g/l $MgSO_4 \times 7\ H_2O$, sterile	Stock solution
Perfusion medium (1x working solution; 1 l per liver)	▪ 50 ml 20x Perfusion medium ▪ 22.5 ml, 7.5% sterile $NaHCO_3$ (w/v) ▪ 2 ml 0.5% sterile phenol red ▪ Fill up to 1,000 ml with sterile, double-distilled water; adjust to pH 7.4 with 1 M NaOH or 1 M HCl.	Basic medium for perfusion of the liver and Collagenase H solution and washing steps.
First perfusion step	Add to the Perfusion medium (1x): ▪ 0.08 g/l (0.2 mM) EGTA, sterile	Step 4
Collagenase H solution (second perfusion step)	Dissolve 100 mg Collagenase H in 4 ml 1x Perfusion medium (final concentration 25 mg/ml) and filter through 0.2 µm filter. ⚠ ***To activate the collagenase, add 2.5 ml sterile 200 mM $CaCl_2$ solution to 100 ml collagenase Perfusion medium.*** ⓘ *4 ml of a 25 mg/ml solution to the Perfusion medium (final concentration approximately 1 mg/ml).*	Step 7
Culture medium	DMEM (1.1 g/l glucose) containing 1x MEM vitamins, 1x MEM amino acids, 1x non-essential amino acids, 2 mM L-glutamine, 1% lactate (pH 7.4), 1% Penicillin-Streptomycin*. ⚠ ***Add 5% fetal calf serum (FCS) immediately before plating the cells.***	Culturing isolated cells.

Serum-free subculture medium	Add to the culture medium: ▪ 1 mg/ml BSA* (bovine serum albumin) ▪ 1 × 10^{-4} mM dexamethasone ▪ 2 × 10^{-5} mM Insulin*	Culturing isolated cells.
Subculture medium with serum	Add to the culture medium: ▪ 5% FCS ▪ 1 × 10^{-4} mM dexamethasone ▪ 2 × 10^{-5} mM Insulin*	Can be used after the first medium exchange, 90 minutes after plating.
Collagen-coating solution	Dissolve 20 mg collagen, such as Collagen from rat tail* in 10 ml sterile acetic acid (final concentration 2 mg/ml) or use sterile collagen solution.	Step 13
Isolation of adipocytes		
Disintegration medium	MEM-Earle containing 5 mM $NaHCO_3$, 20 mM HEPES; adjust to pH 7.8 with 1 M NaOH or 1 M HCl and then add 10% BSA and 2 mg/ml Collagenase H.	Step 4
Buffer solution	PBS containing 5 mM glucose, 100 U/100 µg per ml Penicillin-Streptomycin*.	Step 2
Culture medium	According to the individual requirements, for example, MEM-Earle containing 5 mM $NaHCO_3$, 20 mM HEPES; adjust to pH 7.4 with 1 M NaOH or 1 M HCl and then add 2.5% BSA.	Culturing isolated cells.

2.2. Protocols

Isolation of rat hepatocytes

To obtain a high yield of intact cells (up to 95%), perfusion of the liver with collagenase is the method of choice. The perfusion is carried out in two steps through the hepatic portal vein and involves initially (first step) a calcium-removing solution (Ca^{2+}-free and/or EGTA containing) followed by a collagenase containing medium (second step). Since collagenase is a Ca^{2+}- requiring enzyme, Ca^{2+} should be present during the second step.

1. Anesthetize male Spraque-Dawley rats (body weight approximately 200 g) with 50 µg Nembutal IP/g living weight, and open the abdomen.

2. Spray liver and intestine with approximately 0.5 ml Liquemin to avoid coagulation.

3. Uncover the liver and inject a cannula into the portal vein.

4. Start perfusion (first step) with 1x perfusion medium, Ca^{2+}-free, with a pressure of 50 ml of H_2O.
   – Discard the first 50 ml to remove erythrocytes and plasma proteins.

5. Remove the liver from the body and transfer it into a perfusion apparatus, which maintains the perfusate temperature at +37°C and constantly oxygenates the perfusate.

6. Perfuse the liver (recirculating via backflow through the caval vein) with 100 ml 1x perfusion medium being oxygenated with carbogen (95% O_2, 5% CO_2) at a flow rate of 40 ml/minute for 5 minutes.

7. Add sterile Collagenase H solution (4 ml of a 25 mg/ml solution) to the perfusion medium (final concentration approximately 1 mg/ml), and continue the perfusion for 10 to 30 minutes (second step).
   ⚠ ***Perfusion is terminated when the liver shows a soft consistency (check by finger pressing).***

8. After the perfusion, place the liver into a sterile 50 mm petri dish containing approximately 20 ml 1x perfusion medium.
   – Disrupt the liver capsula with two sterile forceps and dissociate liver cells by gently shaking the liver.

9. Remove the not dissociated tissue pieces and filter the cell suspension through a 74 mm filter or 4 sheets sterile gauze, respectively.

10. Collect the filtrate in a sterile beaker and fill up to 200 ml with 1x perfusion medium (oxygenated with carbogen).

11. Spin the cell suspension in 50 ml portions for 60 seconds (50 × *g*, at +15 to +25°C), and resuspend the cells in 1x perfusion medium (oxygenated with carbogen), reducing the volume by 50%.

12. Repeat this washing procedure twice always reducing the volume by 50%.

13. Resuspend the cells in culture medium containing FCS and seed 3 × 10^5 cells/ml in collagen-coated culture vessels (2.5 µl/cm^2 of a 2 mg/ml collagen-coating solution).
   – Incubate at +37°C with 5% CO_2.

14. Remove culture medium and not adherent cells 90 minutes after plating.

i For further cell culture (subculture), serum-free culture medium can be used.

Isolation of adipocytes

The Collagenase H preparation can also be used to isolate adipocytes from epididymal fat pads of rats.
i With the procedure described below, a yield of approximately 1 × 10^6 cells/g adipose tissue can be obtained.

1. Sacrifice male Wistar rats (150 to 200 g) by cervical dislocation.

2. Remove epididymal tissue and collect it in buffer solution (+4°C) on ice.

3. For the isolation of cells, dissect epididymal fat pads into 4 to 5 pieces.

4. Place 3 to 4 g tissue collected from 2 to 4 animals into plastic tubes, such as 50 ml centrifuge tubes,containing 4 ml disintegration medium.

5. Incubate 30 to 60 minutes at +37°C in a shaking water bath (120/min).
   i Disintegration is finished when the suspension shows a milky-white turbidity.
   ⚠ ***Over digestion results in a destruction of the cell membrane, which can be recognized by the appearance of oil drops on the surface of the cell suspension.***

6. After the incubation, fill the suspension up to 20 ml with culture medium.

7. Filter the suspension through a 74 µm filter or 4 sheets of sterile gauze, respectively.

8. Wash the cells 3 times with culture medium to remove the collagenase solution:
   – Spin the filtered suspension down for 30 seconds at 40 × *g* and discard the lower phase.
   ⚠ ***The adipocytes are floating within the upper phase.***
   – Add approximately 20 ml fresh culture medium and repeat the washing procedure two more times.
   ⚠ ***To avoid irritation of the adipocytes floating within the upper phase, place a silicone tube into the centrifuge tubes before spinning down which makes removing of the lower phase easier.***

9. For further culture, resuspend cells in culture medium and keep the culture vessel at +37°C.

2.3. Parameters

Activator

Ca^{2+}

Biological Activity

Tested for the isolation of hepatocytes from rat liver: <80% viable cells (Trypan Blue exclusion test).
Hepatocytes were isolated by the collagenase perfusion method.

EC-Number

EC 3.4.24.3

Inhibition

EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol.
Collagenase is not inhibited by serum.

pH Optimum

pH 6.0 to 8.0

Specific Activity

0.15 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.

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 Wünsch) under assay conditions (Wünsch E, Heidrich HG, 1963).

Working Concentration

Isolation of rat hepatocytes: approximately 1 mg/ml
Isolation of adipocytes: Approximately 2 mg/ml
Refer to the Certificate of Analysis for the lot-specific concentration.

3. Additional Information on this Product

3.1. Test Principle

Preparation

Collagenase H is prepared from *Clostridium histolyticum* cultures by filtration, ammonium sulfate precipitation, dialysis, and lyophilization.

3.2. References

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

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.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ 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		
Insulin, human	100 mg	11 376 497 001
Penicillin-Streptomycin	for 20 ml, 500x	11 074 440 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001

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.

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