

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



Liberase T-Flex, Research Grade

0.2 µm filtered, lyophilizate

 **Version: 07**

Content Version: November 2020

Partially blended purified enzymes for tissue dissociation

Cat. No. 05 989 132 001 1 kit
1 x 500 mg Collagenase blend
2 x 15 mg Thermolysin

Store the kit at -15 to -25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	Collagenase I/II Blend Research Grade (500 mg)	- White lyophilizate. - Enzyme blend Collagenase I/II and a small quantity of buffer salts.	1 bottle, 500 mg
2	red	Thermolysin Research Grade (15 mg)	- White lyophilizate. - Enzyme Thermolysin and a small quantity of buffer salts.	2 bottles, 15 mg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at -15 to -25°C , the kit is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	Collagenase I/II Blend Research Grade (500 mg)	Store dry at -15 to -25°C .
2	red	Thermolysin Research Grade (15 mg)	

Reconstitution

- Reconstitute the lyophilized enzyme with tissue-dissociation buffer or phosphate buffered saline (PBS).
 - Do not use bacteriostatic water for injection. This type of water contains preservatives that inhibit collagenase enzyme activity.
 - ⚠ Do not add serum or other components that may influence enzyme activity to the dissociation buffer, such as albumin or protease inhibitors. In addition, enzyme stability is reduced at higher concentrations and warmer temperatures ($>+4^{\circ}\text{C}$), therefore, avoid both conditions for any duration of time.**
 - i Reconstitute the entire vial. Do not weigh individual aliquots of the lyophilizate. The introduction of moisture into the vial results in a decline in enzymatic activity.**

Vial Content	Reconstitution Volume [ml]	Collagenase Wünsch Unit Concentration [U/ml]	Enzyme Concentration [mg/ml]
Collagenase	20	130	25
Thermolysin	15	-	1.0

Place vial on ice to rehydrate the lyophilized enzyme.– Gently agitate the vial at $+2$ to $+8^{\circ}\text{C}$ until enzyme is completely dissolved (maximum 30 minutes).

Depending on the type of tissue-dissociation buffer used to dissolve Liberase Research Grade Purified Enzyme Blends, slight precipitations may be observed which are dissolved in the diluted working solution and have no influence on enzyme activity.

Remove an aliquot of the stock solution to prepare the working solution, see section, Working Concentration.

1.3. Additional Equipment and Reagent required

For reconstitution of lyophilisate

- Tissue dissociation buffer, or
- Phosphate buffered buffer (PBS)

⚠ Do not add components that may influence enzyme activity (e.g., albumin, serum, or protease inhibitors) to the dissociation buffer.

1.4. Application

The Liberase T-Flex Research Grade kit enables to make individual blends of collagenase and Thermolysin ratios. As a guideline please refer to the ratios described in the section “Target Activities” (Section **General Considerations**). Mix different ratio of neutral protease activity, relative to the collagenase activity to get an enzyme blend appropriate for your application.

Product Description

The Liberase T-Flex Research Grade kit contains highly purified collagenase class I and class II from *Clostridium histolyticum*. The two collagenase isoforms are blended in a precise ratio with each other. Highly purified thermolysin from *Bacillus thermoproteolyticus* is provided in a separate vial to allow individual adjustments of enzyme ratios for optimization of your tissue dissociation protocol.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Target Activities

Each vial of Liberase T-Flex Research Grade purified enzyme is filled by total protein mass. Combined collagenolytic activity of the Collagenase I and II isoforms is measured by the method of Wünsch (1). Neutral protease activity is measured by a non-fluorescent Casein assay (2). The kit components allow to make individual blends of Collagenase and Thermolysin ratios.

Composition of Liberase T-Flex Research Grade Purified Enzyme Blends

Product	Target Enzyme Mix [mg]	Target Collagenase Activity [Wünsch U/ vial]	Target Neutral	Enzyme Mixture	Main Application
Collagenase I/II Blend Research Grade Thermolysin Research Grade	500 15	2600	Low	+(+)	Very gentle isolation of islets from pancreas
Collagenase I/II Blend Research Grade Thermolysin Research Grade	500 24	2600	Low	++	Isolation of islets from rodent pancreas
Collagenase I/II Blend Research Grade Thermolysin Research Grade	500 30	2600	Low	++(+)	More harsh isolation of islets from pancreas

⁽¹⁾ + equals lowest neutral protease activity/mg protein and +++++ equals highest neutral protease activity/mg protein.

Optimization of tissue dissociation protocol

This section will help you interpret your tissue dissociation results, and find opportunities to improve your cell yield, viability, and/or functionality. Before continuing, see section, **Protocols, Factors affecting Liberase Research Grade selection** regarding enzyme requirements for tissue dissociation, as well as the points below:

- Liberase T-Flex Research Grade Purified Enzyme Blends contain only collagenase and neutral protease.
- Collagenase enzymes digest the intercellular matrix.
- Neutral proteases act synergistic with collagenase.
- Given sufficient time and concentration, neutral proteases damage cell surface proteins.
- Time of dissociation, enzyme ratios, and enzyme concentration all affect the tissue-dissociation outcome.
- Use Liberase T-Flex Research Grade without modifying factors, such as serum, BSA, or protease inhibitors.

Use the following table for optimization in the sequence provided.

- 1 Note whether the yield, viability, or functionality of your cells isolated with Liberase Research Grade Purified Enzyme Blend is less than optimal.
- 2 Find the probable cause, then act on the recommendation.
- 3 Refer to enzyme mixture aggressiveness described in section, **Target activities** for information on neutral protease specific activity increasing within the Liberase Research Grade panel.

2. How to Use this Product

Observation 1	Observation 2	Possible Cause	Recommendation
Low cell viability.	Dissociation very rapid.	Enzyme concentration too high.	Reduce enzyme concentration by 50%.
		Enzyme mixture aggressiveness too high.	Select a Liberase Research Grade Purified Enzyme Blend containing lower amounts of neutral protease.
	Dissociation very slow.	Enzyme concentration too low.	Increase enzyme concentration by 50%.
		Enzyme mixture aggressiveness too low.	Select a Liberase Research Grade Purified Enzyme Blend containing higher amounts of neutral protease.
Impaired cell function.	Cell viability >80%; cell yield is reasonable.	Enzyme concentration too high.	Reduce enzyme concentration by 25%.
		Enzyme mixture aggressiveness too high.	Select a Liberase Research Grade Purified Enzyme Blend containing lower amounts of neutral protease.
Low cell yield.	Cell viability >80%.	Enzyme concentration too low.	Increase enzyme concentration by 25 to 50%.
		Enzyme mixture aggressiveness too low.	Select a Liberase Research Grade Purified Enzyme Blend containing higher amounts of neutral protease.
	Cell viability <80%.	Enzyme concentration too high.	Reduce enzyme concentration by 50%.
		Enzyme mixture aggressiveness too high.	Select a Liberase Research Grade Purified Enzyme Blend containing lower amounts of neutral protease.
		Mechanical (shear) force is excessive	Reduce shear force in all aspects of dissociation. Treat tissue gently.
Released cells clump in gelatinous stringy form.	Cell yield and viability are acceptable	DNA release subsequent to cell lysis, causes clumping.	More prevalent in some tissues. If cell viability is acceptable, add DNase to dissociation mixture.
	Cell yield or viability are reduced.	Mechanical (shear) force is excessive.	Reduce shear force in all aspects of dissociation. Treat tissue gently.

Safety Information

Precautions

- Protect respiratory system, eyes, and skin when handling proteases.
- Open the vials inside a laminar flow hood.

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.

2.2. Protocols

Factors affecting Liberase Research Grade selection

Prior to choosing a Liberase Research Grade for your application, familiarize yourself with the factors that influence enzyme requirements. Enzyme requirements for tissue dissociation are determined by:

- Tissue type
- Species
- Dissociation protocol
- Desired outcome of tissue dissociation

⚠ Liberase Research Grade Purified Enzyme Blends are formulated for use with most calcium-containing buffers. However, protease inhibitors, serum, and BSA will inhibit Liberase Research Grade performance, therefore, they must be excluded from the dissociation.

2.3. Parameters

Cofactors

Zinc, Calcium

Inhibition

Purified collagenase contains approximately 1 mole of zinc and 2 to 7 moles of calcium per mole of enzyme. Exposure of the enzyme to divalent cation chelators removes zinc and calcium, thus rendering the enzyme inactive. Liberase Enzyme Blends are inhibited by:

- 0.1 M EDTA
- Cysteine
- Mercaptoethanol
- Protease inhibitors
- Serum
- Albumin

pH Optimum

In general, the optimum pH for tissue dissociation is the one which is physiologically appropriate for the cells to be isolated (pH 7.4).

i *Liberase Research Grade Purified Enzyme Blends are mixtures of enzymes that act differently upon different substrates. Plots of in vitro enzyme activity versus pH (measured with artificial substrates) cannot predict the effects of pH on tissue dissociation.*

Stabilizers

Calcium

Temperature Optimum

For general tissue dissociation, use a temperature range of +35 to +37°C. Lower temperatures will reduce enzyme activity and the rate of tissue dissociation.

i *Liberase Research Grade Purified Enzyme Blends are mixtures of enzymes that act differently upon different substrates. Plots of in vitro enzyme activity versus temperature (measured with artificial substrates) cannot predict the effects of temperature on tissue dissociation.*

Working Concentration

All Liberase Research Grade Purified Enzyme Blends have substantially higher specific activities than traditional collagenases. This means that identical working concentrations of Liberase Research Grade and traditional collagenase, expressed in mg/ml, yield very different effective enzyme concentrations. The goal of this section is to estimate the best starting concentration of Liberase Research Grade to use. This is only a first step due to differences in procedure and lot-to-lot differences in traditional collagenase. After working with this starting concentration, see section, General Considerations, Optimization of tissue-dissociation protocol to find the best enzyme concentration, based upon your experimental needs.

Collagenase specific activity

Collagenase is traditionally diluted to a concentration expressed in mg/ml. Significant lot-to-lot differences in traditional collagenase specific activity require that you establish a new working concentration each time you change lots. This is not the case with Liberase Research Grade Purified Enzymes Blends. Each Liberase Research Grade Purified Enzyme Blend is blended from highly purified enzymes. It is essential to express collagenase concentration in Wünsch U/ml, instead of mg/ml.

i For consistency in your protocol, always express collagenase concentration in terms of enzyme units per milliliter (U/ml).

Convert collagenase specific activity to Wünsch U/mg

Use the following table to convert the collagenase enzyme activity of your current collagenase to Wünsch (collagenase) U/mg. This table calculates Wünsch U/mg from either FALGPA U/mg, or collagen degrading U/mg (Mandl U; CDU).

i These conversions are a reasonable approximation, based upon the expected precision of the different collagenase assays.

Convert from[U/mg]	To[U/mg]	Divide	Example
FALGPA	Wünsch	by 3.9	$3.5 \text{ FALGPA U/mg} \div 3.9 = 0.9 \text{ Wünsch U/mg}$
CDU (Mandl)	Wünsch	by 1,000	$200 \text{ CDU/mg} \div 1,000 = 0.2 \text{ Wünsch U/mg}$

Collagenase working concentration

To estimate a working concentration of Liberase Research Grade, multiply your previous collagenase working concentration (mg/ml) by its specific activity (Wünsch U/mg), see section, Specific Activity, to obtain Wünsch units/ml. To determine how much Liberase Research Grade to use, first multiply your collagenase working concentration (in Wünsch U/ml) times the total volume of your working enzyme solution to obtain the total collagenase activity needed (Wünsch units). Divide the total collagenase activity required by the Liberase Research Grade stock concentration, see section, Reconstitution. This will tell you how many milliliters of Liberase Research Grade stock solution to use in your working enzyme solution.

3. Troubleshooting

Problem	Possible cause	Recommendation
Prolonged dissociation time or incomplete dissociation.	Enzyme decay.	Follow appropriate storage conditions, see section, Storage Conditions (Product) and Storage Conditions (Working Solution) .
	Inappropriate Enzyme reconstitution time.	Follow appropriate reconstitution conditions, see section, Reconstitution .
	Inappropriate Enzyme dilution.	Verify dilution.
	Enzyme inhibition or tissue exposed to enzyme inhibitors.	Check for presence of inhibitors in all buffers, see section, Inhibition .
	Incubation temperature too low.	Verify +37°C is incubation temperature.
Low cell viability and yield.	Tissue stored at elevated temperature prior to dissociation.	Reduce time and temperature of ischemia.
	Prolonged tissue ischemia time.	Reduce time of tissue ischemia.
	Incubation time too long.	Reduce incubation time.
	Inappropriate Research Grade dilution.	Verify dilution.
	Incubation temperature too high.	Verify +37°C is incubation temperature.
Decreased cell viability or <i>in vitro</i> survival.	Endotoxin exposure.	Check all tissue dissociation reagents for endotoxin contamination.
Liberase Research Grade does not go into solution within 30 minutes.	The volume used for the reconstitution of the lyophilized enzyme is too low.	Increase volume of reconstitution buffer two-fold.

4. Additional Information on this Product

4.1. Test Principle

How this product works

Liberase T-Flex Research Grade kit contains enzymes of purified collagenase isoforms I and II, and the neutral protease Thermolysin. The collagenase isoforms are purified from the fermentation of *Clostridium histolyticum*. Thermolysin is purified from the fermentation of *Bacillus thermoproteolyticus*. The methods for purification and blending of these components are described in one or more of the following patent numbers: US 5,753,485 and US 5,830,741.

4.2. Quality Control

Several tests are performed on Liberase Research Grade Purified Enzyme Blends prior to release for sale. The following table shows the acceptance ranges.

Product	Pack Size [mg]	Collagenase Content [mg/vial] ⁽¹⁾	Collagenase I Integrity ⁽¹⁾	Endotoxin [EU/mg] ⁽²⁾
Collagenase I/II Blend Research Grade	500	430 - 644	≥ 10% area	≤ 10
Thermolysin Research Grade	15	12.0 - 18.0	-	≤ 50

⁽¹⁾ Measured by High Performance Liquid Chromatography (HPLC) analysis.

⁽²⁾ Measured by a standard Limulus Amebocyte Lysate (LAL) assay.

i The actual values determined for each lot are available in the respective Certificates of Analysis.

Measurement of collagenase I and collagenase II integrity by HPLC

Almost no fragmentation of both collagenase enzymes can be detected in Liberase TM Research Grade blends, proving the purity of this enzyme mixture (Fig 1).

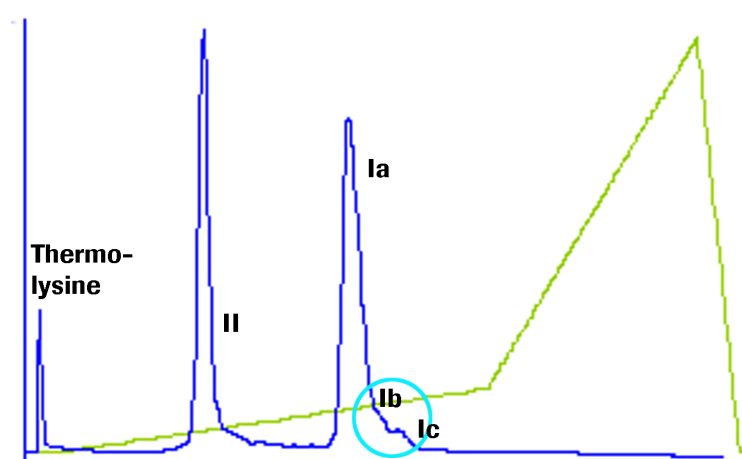


Fig. 1: HPLC analysis of Liberase TM Research Grade

5. Supplementary Information

5.1. 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.
① ② ③ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.

Editorial changes.

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

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Liberase Research Grade Purified Enzyme Blends	Liberase DL Research Grade (Dispase Low), 10 mg (2 x 5 mg)	05 401 160 001
	Liberase DH Research Grade (Dispase High), 10 mg (2 x 5 mg)	05 401 054 001
	Liberase TL Research Grade (Thermolysin Low), 10 mg (2 x 5 mg)	05 401 020 001
	Liberase TM Research Grade (Thermolysin Medium), 10 mg (2 x 5 mg)	05 401 119 001
	Liberase TH Research Grade (Thermolysin High), 10 mg (2 x 5 mg)	05 401 135 001
	Liberase DL Research Grade (Dispase Low), 100 mg (2 x 50 mg)	05 466 202 001
	Liberase DH Research Grade (Dispase High), 100 mg (2 x 50 mg)	05 401 089 001
	Liberase TM Research Grade (Thermolysin Medium), 100 mg (2 x 50 mg)	05 401 127 001
	Liberase TH Research Grade (Thermolysin High), 100 mg (2 x 50 mg)	05 401 151 001

5.4. Trademarks

LIBERASE is a trademark of Roche.

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

5.5. License Disclaimer

For patent license limitations for individual products please refer to:

<http://technical-support.roche.com>.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. Contact and Support

For more information about this product, as well as documentation such as Instructions for Use and Safety Data Sheets, please visit custombiotech.roche.com.

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