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

# Liberase Research Grade Purified Enzyme Blends

**Version: 08**
Content Version: December 2020

Blended purified enzymes for tissue dissociation

| | |
|---|---|
| **Cat. No. 05 401 160 001** | Liberase DL Research Grade (Dispase Low)<br>10 mg (2 x 5 mg) |
| **Cat. No. 05 401 054 001** | Liberase DH Research Grade (Dispase High)<br>10 mg (2 × 5 mg) |
| **Cat. No. 05 401 020 001** | Liberase TL Research Grade (Thermolysin Low)<br>10 mg (2 × 5 mg) |
| **Cat. No. 05 401 119 001** | Liberase TM Research Grade (Thermolysin Medium)<br>10 mg (2 × 5 mg) |
| **Cat. No. 05 401 135 001** | Liberase TH Research Grade (Thermolysin High)<br>10 mg (2 × 5 mg) |
| **Cat. No. 05 466 202 001** | Liberase DL Research Grade (Dispase Low)<br>100 mg (2 x 50 mg) |
| **Cat. No. 05 401 089 001** | Liberase DH Research Grade (Dispase High)<br>100 mg (2 × 50 mg) |
| **Cat. No. 05 401 127 001** | Liberase TM Research Grade (Thermolysin Medium)<br>100 mg (2 × 50 mg) |
| **Cat. No. 05 401 151 001** | Liberase TH Research Grade (Thermolysin High)<br>100 mg (2 × 50 mg) |

**Store lyophilizates at −15 to −25°C.**

**1. General Information ..... 3**

1.1. Contents ..... 3

1.2. Storage and Stability ..... 3
Storage Conditions (Product) ..... 3
Storage Conditions (Working Solution) ..... 4
Reconstitution ..... 4

1.3. Additional Equipment and Reagent required ..... 4

1.4. Application ..... 4

**2. How to Use this Product ..... 5**

2.1. Before you Begin ..... 5
General Considerations ..... 5
Target activities ..... 5
Optimization of tissue dissociation protocol ..... 5
Safety Information ..... 6
Precautions ..... 7
Laboratory procedures ..... 7
Waste handling ..... 7

2.2. Protocols ..... 7
Factors affecting Liberase Research Grade selection ..... 7
Select a Liberase Research Grade ..... 7

2.3. Parameters ..... 8
Cofactors ..... 8
Inhibition ..... 8
pH Optimum ..... 8
Stabilizers ..... 8
Temperature Optimum ..... 8
Working Concentration ..... 8
Collagenase specific activity ..... 8
Convert collagenase specific activity to Wünsch U/mg ..... 9
Collagenase working concentration ..... 9

**3. Troubleshooting ..... 9**

**4. Additional Information on this Product ..... 10**

4.1. Test Principle ..... 10
How this product works ..... 10

4.2. References ..... 10

4.3. Quality Control ..... 10

**5. Supplementary Information ..... 10**

5.1. Conventions ..... 10

5.2. Changes to previous version ..... 10

5.3. Trademarks ..... 11

5.4. License Disclaimer ..... 11

5.5. Regulatory Disclaimer ..... 11

5.6. Safety Data Sheet ..... 11

5.7. Contact and Support ..... 11

# 1. General Information

## 1.1. Contents

| Label | Function / Description | Catalog Number | Content |
|---|---|---|---|
| Liberase DL Research Grade | White lyophilizate, containing blended enzymes and a small quantity of buffer salts. | 05 401 160 001 | 2 vials, 5 mg each |
| | | 05 466 202 001 | 2 vials, 50 mg each |
| Liberase DH Research Grade | White lyophilizate, containing blended enzymes and a small quantity of buffer salts. | 05 401 054 001 | 2 vials, 5 mg each |
| | | 05 401 089 001 | 2 vials, 50 mg each |
| Liberase TL Research Grade | White lyophilizate, containing blended enzymes and a small quantity of buffer salts. | 05 401 020 001 | 2 vials, 5 mg each |
| Liberase TM Research Grade | White lyophilizate, containing blended enzymes and a small quantity of buffer salts. | 05 401 119 001 | 2 vials, 5 mg each |
| | | 05 401 127 001 | 2 vials, 50 mg each |
| Liberase TH Research Grade | White lyophilizate, containing blended enzymes and a small quantity of buffer salts. | 05 401 135 001 | 2 vials, 5 mg each |
| | | 05 401 151 001 | 2 vials, 50 mg each |

## 1.2. Storage and Stability

### Storage Conditions (Product)

The product is shipped on dry ice.
When stored at −15 to −25°C, the lyophilizates are stable through the expiry date printed on the box label.
⚠ ***The expiration date is not printed on the individual vials.***

| Label | Storage |
|---|---|
| Liberase DL | Store dry at −15 to −25°C. |
| Liberase DH | |
| Liberase TL | |
| Liberase TM | |
| Liberase TH | |

## Storage Conditions (Working Solution)

Store unused stock solution in single-use aliquots at −15 to −25°C.
⚠ ***Avoid repeated freezing and thawing.***

## Reconstitution

1. Reconstitute the lyophilized enzyme with injection-quality sterile water or tissue-dissociation buffer.
   – 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°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 Size | Reconstitution Volume [ml] | Collagenase Wünsch Unit Concentration [U/ml] | Total Collagenase Concentration [mg/ml] |
|---|---|---|---|
| Small | 2 | 13 | 2.5 |
| Large | 10 | 26 | 5.0 |

2. Place vial on ice to rehydrate the lyophilized enzyme.
   – Gently agitate the vial at +2 to + 8°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.***

3. 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 lyophilizates

- Injection-quality sterile water or tissue-dissociation buffer

## 1.4. Application

Liberase Research Grade Purified Enzyme Blends are mixtures of highly purified collagenase and neutral protease enzymes, formulated for efficient, gentle, and reproducible dissociation of tissue from a wide variety of sources. The purified collagenase enzymes are isoforms I and II, as specified by the nomenclature of Bond and Van Wart. The target substrates for these enzyme blends are the collagen and non-collagen proteins that comprise the intercellular matrix.

# 2. How to Use this Product

## 2.1. Before you Begin

## General Considerations

### Target activities

The principle difference between the Liberase Research Grade Purified Enzyme Blends is the amount of neutral protease activity, relative to its collagenase activity. Each vial of Liberase Research Grade Purified Enzyme Blend is filled by total protein mass. Combined collagenolytic activity of the collagenase I and II isoforms is measured by the method of Wünsch (Wünsch E, Heidrich HG, 1963). Neutral protease activity is measured by a non-fluorescent casein assay.

#### Composition of Liberase Research Grade Purified Enzyme Blends

| Product | Target Collagenase Content [mg/vial] | Target Collagenase Activity [Wünsch U/vial] | Target Neutral Protease Amount | Enzyme Mixture Aggressiveness | Neutral Protease Type | Endotoxin [EU/mg] |
|---|---|---|---|---|---|---|
| Liberase DL Research Grade | 5 | 26 | Low | +[1] | Dispase® | ≤50 |
| | 50 | 260 | | | | |
| Liberase DH Research Grade | 5 | 26 | High | +++ | Dispase® | ≤50 |
| | 50 | 260 | | | | |
| Liberase TL Research Grade | 5 | 26 | Low | ++ | Thermolysin | ≤50 |
| Liberase TM Research Grade | 5 | 26 | Medium | ++++ | Thermolysin | ≤50 |
| | 50 | 260 | | | | |
| Liberase TH Research Grade | 5 | 26 | High | +++++[2] | Thermolysin | ≤50 |
| | 50 | 260 | | | | |

[1] + equals lowest neutral protease activity/mg protein.
[2] +++++ 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 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 Research Grade Purified Enzyme Blends without modifying factors, such as serum, BSA, or protease inhibitors.

## 2. How to Use this Product

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.

| 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

## Select a Liberase Research Grade

| If... | Then... |
|---|---|
| you are dissociating a tissue with which you have no previous experience, | use Liberase TM Research Grade which has the greatest overall range of applicability and is a good starting point for the dissociation of many tissues. The Liberase Research Grade panel offers a continuous range of “Enzyme Mixture Aggressiveness”. Liberase DL Research Grade is the most gentle (lowest protease activity per mg of protein), and Liberase TH Research Grade is the most aggressive (highest neutral protease activity per mg protein). For additional information, see section, **General Considerations, Target activities**.<br>*Use Liberase TM Research Grade at a concentration of 0.08 to 0.28 Wünsch U/ml.* |
| you have previously used collagenase for this application, | in most cases, you can select a Liberase Research Grade based upon your previous experience with traditional collagenase.<br>*To estimate the working concentration, see section,* **Working Concentration**.<br>After selecting a Liberase Research Grade and corresponding starting concentration, apply it to your protocol. For additional information on optimization, review the optimization table in section, **General Considerations**. |
| you have not previously isolated cells from tissue through enzymatic digestion, | see basic cell culture publications for background information; then refer to section, **Working Concentration**. |

⚠ ***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).

*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 FALGPA U/mg ÷ 3.9 = 0.9 Wünsch U/mg |
| CDU (Mandl) | Wünsch | by 1,000 | 200 CDU/mg ÷ 1,000 = 0.2 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 *in vitro* 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 Research Grade Purified Enzymes are blends of purified collagenase isoforms I and II, and a neutral protease.

- The collagenase isoforms are purified from the fermentation of *Clostridium histolyticum*.
- Liberase DL and Liberase DH Research Grade contain the neutral protease Dispase®, which is purified from *Bacillus polymyxa* fermentation.
- Liberase TL, TM, and TH Research Grade contain the neutral protease thermolysin, which is purified from the fermentation of *Bacillus thermoproteolyticus*.

## 4.2. References


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


## 4.3. Quality Control

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

# 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. |
| 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. |

## 5.2. Changes to previous version

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

## 5.3. Trademarks

LIBERASE is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

## 5.4. License Disclaimer

For patent license limitations for individual products please refer to:
**List of biochemical reagent products**.

## 5.5. Regulatory Disclaimer

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

## 5.6. Safety Data Sheet

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

## 5.7. 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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