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Not for use in diagnostic procedures.

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Liberase Research Grade Purified Enzyme Blends

Version 06

Content version: August 2013

Blended purified enzymes for tissue dissociation

	Cat. Nos.	Pack size (Total Collagenase amount)
Liberase DL Research Grade (Dispase® Low)	**05 401 160 001** **05 466 202 001**	10 mg (2 × 5 mg) 100 mg (2 × 50 mg)
Liberase DH Research Grade (Dispase® High)	**05 401 054 001** **05 401 089 001**	10 mg (2 × 5 mg) 100 mg (2 × 50 mg)
Liberase TL Research Grade (Thermolysin Low)	**05 401 020 001**	10 mg (2 × 5 mg)
Liberase TM Research Grade (Thermolysin Medium)	**05 401 119 001** **05 401 127 001**	10 mg (2 × 5 mg) 100 mg (2 × 50 mg)
Liberase TH Research Grade (Thermolysin High)	**05 401 135 001** **05 401 151 001**	10 mg (2 × 5 mg) 100 mg (2 × 50 mg)
Liberase Research Grade Selection Kit	**05 401 046 001**	25 mg (5 × 5 mg)

Each Liberase Research Grade Purified Enzyme Blend contains 2 vials. The Liberase Research Grade Selection Kit contains 5 vials.

Store Liberase Research Grade Purified Enzyme Blends at −15 to −25°C

www.roche-applied-science.com

Table of Contents

1. What this Product Does

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 (3). The target substrates for these enzyme blends are the collagen and non-collagen proteins that comprise the intercellular matrix.

Formulation

Liberase Research Grade Purified Enzyme Blends are white lyophilizates consisting of blended enzymes, and a small quantity of buffer salts.

Enzyme Characteristics:

Target Activities

The composition of each Liberase Research Grade Purified Enzyme Blend is described in the following table. The principle difference between the products is the amount of neutral protease activity, relative to its collagenase activity.

Each vial of Liberase Research Grade Purified Enzme Blend 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).

Product	Target Collagenase Content (mg/vial)	Target Collagenase Activity (Wünsch units/vial)	Target Neutral Protease Amount	Enzyme Mixture Aggressiveness	Neutral Protease Type	Endotoxin (EU/mg)
Liberase DL Research Grade	5 50	26 260	Low	+	Dispase®	≤50
Liberase DH Research Grade	5 50	26 260	High	+++	Dispase®	≤50
Liberase TL Research Grade	5 50	26 260	Low	++	Thermolysin	≤50
Liberase TM Research Grade	5 50	26 260	Medium	++++	Thermolysin	≤50
Liberase TH Research Grade	5 50	26 260	High	+++++	Thermolysin	≤50
Liberase Research Grade Selection Kit	5 each	26 each	See above	See above	See above	≤50 each

\+ = (lowest neutral protease activity/mg protein)
+++++ = (highest neutral protease activity/mg protein)

Storage and Stability

- The lyophilized enzyme is stable at −15 to −25°C until the expiration date printed on the label of the box.
- Liberase Research Grade Purified Enzyme Blends are shipped on dry ice.
- Store in a dry environment.

⚠ Please note that the expiration date is not printed on the individual vials.

Temperature and pH

pH Optimum	Temperature Optimum
▪ In general, the optimal pH for tissue dissociation is the one which is physiologically appropriate for the cells to be isolated (pH 7.4).	▪ For general tissue dissociation, use a temperature range of +35 to +37°C. The usage of lower temperatures will reduce enzyme activity (and the rate of tissue dissociation).

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

Modifying Factors

Purified collagenase contains approximately 1 mole of zinc and 2 to 7 moles of calcium per mole of enzyme (3). Exposure of the enzyme to divalent cation chelators removes zinc and calcium, thus rendering the enzyme inactive (4).

Modification	Factor
Inhibitor	0.1 M EDTA (5) Cysteine (4) Mercaptoethanol (4) Protease inhibitors Serum Albumin
Stabilizer	Calcium (3)
Cofactor	Zinc, Calcium (3)

2. How to Use this Product

2.1 Before You Begin

Reconstitution and Storage

⚠ Do not use bacteriostatic water for injection. This type of water contains preservatives that inhibit collagenase enzyme activity.

⚠ Please protect respiratory system, eyes and skin when handling proteases. Open the vials inside a laminar flow hood.

❶ Reconstitute the lyophilized enzyme with injection-quality sterile water or tissue-dissociation buffer. Do not add serum or other components that may influence enzyme activity (*e.g.,* albumin or protease inhibitors) to the dissociation buffer. Enzyme stability is reduced at higher concentrations and warmer temperatures (> 4°C). Therefore, avoid both conditions for any duration of time.

🔍 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	Small	Large
Reconstitution volume	2 ml	10 ml
Collagenase Wünsch units/ml	13	26
Total Collagenase Concentration [mg/ml]	2.5	5.0

❷ Place vial on ice to rehydrate the lyophilized enzyme.

❸ Gently agitate the vial at +2 to + 8°C until enzyme is completely dissolved (max. 30 min).

⚠ 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.

❹ Remove an aliquot of the stock solution to prepare the working solution (see sections 2.3 and 2.6).

❺ Store unused stock solution in single-use aliquots at –15 to –25°C. For further information on product stability please visit our Liberase Enzyme website at **www.collagenase.com**.

⚠ **Avoid repeated freezing and thawing.**

2.2 How to Select the Best Liberase Purified Enzyme Blend for Your Application

Factors Affecting Liberase Research Grade Selection

Prior to choosing a Liberase Research Grade for your application, you must be familiar 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	refer to section 2.3, “Select a Liberase Research Grade for a new procedure”.
you have previously used a Liberase Enzyme 1st generation for this application	refer to section 2.4, “Select a Liberase Research Grade to replace a 1st generation Liberase Enzyme blend”.
you have previously used collagenase for this application	refer to section 2.5, “Select a Liberase Research Grade to replace traditional collagenase”.
you have not previously isolated cells from tissue through enzymatic digestion	refer to a basic cell culture text (6,7,8) for background information, then refer to section 2.6, “Liberase Research Grade Purified Enzyme Blend 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 Select a Liberase Research Grade for a New Procedure

Select a Liberase Research Grade based on Application

Visit our Liberase Research Grade Purified Enzyme Blend website at **www.collagenase.com** to learn if we have identified a Liberase Research Grade Purified Enzyme Blend for your specific application. If your application is not included in our current database, please go to the next section, "Liberase TM Research Grade."

Liberase TM Research Grade

Liberase TM Research Grade 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 more information, go to the section labeled "Enzyme Mixture Aggressiveness" (Chapter 1).

Liberase Research Grade Enzyme Working Concentration

All Liberase Research Grade Purified Enzyme Blends have substantially higher specific activities than traditional collagenases. This means that the working concentration of Liberase Research Grade Purified Enzyme Blends, expressed in mg/ml, will be much lower than that of traditional collagenase.

If . . .	then . . .
your application is included in our list of applications	use the Liberase Research Grade concentration recommended in that application
your application is **NOT** included in our list of applications	use Liberase TM Research Grade at a concentration of 0.08 – 0.28 Wünsch units/ml

Optimization

After selecting a Liberase Research Grade Purified Enzyme Blend and concentration, apply it to your procedure. Optimize your protocol by reviewing the optimization table (section 2.7).

2.4 Select a Liberase Research Grade to Replace a 1st Generation Liberase Enzyme Blend

You can select a Liberase Research Grade based upon your previous experience with Liberase Blendzymes of the 1st generation. Visit our Liberase Research Grade Purified Enzyme Blends website at **www.collagenase.com** for information how to replace your current collagenase:

- Type of Liberase Blend used previously
- Previously used working concentration (mg/ml or Wünsch units/ml)

The mg/vial amounts indicated for the Liberase Blendzymes of the 1st generation referred to whole protein amounts (collagenase I + collagenase II + neutral protease). The mg/vial indications of second generation Liberase Research Grade Purified Enzyme Blends refer to the total collagenase amount without considering the protein amount of neutral protease. Therefore, it is essential to express collagenase concentration of the first generation product in Wünsch units/ml, instead of mg/ml. Wünsch units/ml of 1st and 2nd generation products are equivalent.

Please note that the second generation Liberase Research Grade Purified Enzyme Blends show considerably higher purity of the collagenase I + II compared to the former Liberase Blendzymes. Therefore, the incubation time should be adapted to each application specifically. In most of the cases, a reduction of the incubation time of 10 – 30% will produce optimal results.

2.5 Select a Liberase Research Grade to Replace Traditional Collagenase

Select Liberase Research Grade based on Application

Visit our Liberase Research Grade Purified Enzyme Blends website at **www.collagenase.com** to learn if we have identified a Liberase Research Grade for your specific application. If your application is not included in our current database, please go to the next step, "Select Liberase Research Grade based on current collagenase."

Select Liberase Research Grade based on Current Collagenase

In most cases, you can select a Liberase Research Grade based upon your previous experience with traditional collagenase.

Determine Liberase Research Grade 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. To estimate a working concentration of Liberase Research Grade, go to section 2.6, "Liberase Research Grade Purified Enzyme Blend working concentration."

Optimize Protocol

After selecting a Liberase Research Grade and corresponding starting concentration, apply it to your procedure. Optimize your protocol by reviewing the optimization table (section 2.7).

2.6 Liberase Research Grade Enzyme Working Concentration

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, consult the optimization table (section 2.7) 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 units/ml, instead of mg/ml.

For consistency in your protocol, always express collagenase concentration in terms of enzyme units per ml.

Convert Collagenase Specific Activity to Wünsch units/mg

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

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

To Convert from	To	Divide	Example
FALGPA units/mg	Wünsch units/mg	FALGPA units/mg by 3.9	3.5 FALGPA units/mg ÷ 3.9 = 0.9 Wünsch units/mg
CDU/mg or Mandl units/mg	Wünsch units/mg	CDU/mg by 1000	200 CDU/mg ÷ 1000 = 0.2 Wünsch units/mg

Collagenase Working Concentration

Multiply your previous collagenase working concentration (mg/ml) by its specific activity (Wünsch units/mg, [as determined above]), to obtain Wünsch units/ml. To determine how much Liberase Research Grade to use, first multiply your collagenase working concentration (in Wünsch units/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 step 1 in "Reconstitution and Storage"). This will tell you how many milliliters of Liberase Research Grade stock solution to use in your working enzyme solution.

Optimization

After selecting a Liberase Research Grade and concentration, apply it to your procedure. Optimize your protocol by reviewing the optimization table (section 2.7).

2.7 Optimize Your Tissue Dissociation Procedure

This section will help you interpret your tissue dissociation results, and find opportunities to improve your cell yield, viability, and/or functionality. Before continuing, refer to the points in section 2.2 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 synergistically 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.

Optimization Table

Use the following table in the sequence provided. Note whether the yield, viability, or functionality of your cells isolated with Liberase Research Grade Purified Enzyme Blend is less than optimal. Find the probable cause, then act on the recommendation.

Refer to enzyme mixture agressiveness described in "Target Activities" (Chapter 1) 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.

Observation 1	Observation 2	Possible Cause	Recommendation
Low cell yield	Cell viability >80%	Enzyme concentration too low	Increase enzyme concentration by 25 – 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.

3. Troubleshooting

If you are unable to optimize a Liberase Research Grade for your procedure, or if you encounter difficulties in effective cell isolation, refer to the following table:

	Possible Cause	Recommendation
Prolonged dissociation time or incomplete dissociation	Enzyme decay	Follow appropriate storage conditions (Chapter 1).
	Inappropriate Enzyme reconstitution time	Follow appropriate reconstitution conditions (Chapter 2.1).
	Inappropriate Enzyme dilution	Verify dilution.
	Enzyme inhibition or tissue exposed to enzyme inhibitors	Check for presence of inhibitors in all buffers (Chapter 1).
	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		Increase volume of reconstitution buffer two-fold.

4. Additional Information on this Product

4.1 How this Product Works

How this Product is Purified and Blended

Liberase Research Grade Purified Enzymes are blends of purified collagenase isoforms I and II (3), 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

Target Activities

1 Wünsch, E. & Heidrich, H.G. (1963) Zur quantitativen Bestimmung der Kollagenase. *Z. Physiol. Chem*. **333**, 149.

2 Matsubara, H. (1970) Purification and assay of thermolysin. *Methods in Enzymology* **19**, 642-651.

Modifying Factors

3 Bond, M.D. & Van Wart, H.E. (1984) Characterization of the individual collagenases from *Clostridium histolyticum*. *Biochemistry* **23**, 3085-3091.

4 Seifter, S. & Gallop, P.M. (1960) Collagenase from *Clostridium histolyticum*. *Methods in Enzymology* **5**, 659-665.

5 McShane, P., *et al.* (1989) Protease activity in pancreatic islet isolation by enzymatic degradation. *Diabetes* **38** (Suppl. 1), 126-128.

Basic Tissue Culture

6 Pollard, J.W., & Walker, J.M. (1997) Basic Cell Culture Protocols, Humana Press.

7 Jones, G.E. (1996) Human Cell Culture Protocols, Humana Press.

8 Freshney, R.I. (2005) Culture of Animal Cells: A Manual of Basic Technique, Wiley & Sons.

4.3 Quality Control

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

Product	Pack size	Collagenase content (mg/vial) [1]	Collagenase I integrity [1]	Endotoxin (EU/mg) [2]
Liberase DL Research Grade	10 mg	3.2 – 6.0	≥60 %	≤50
	100 mg	33 – 60		
Liberase DH Research Grade	10 mg	3.0 – 6.0	≥60 %	≤50
	100 mg	30 – 60		
Liberase TL Research Grade	10 mg	4.0 – 6.0	≥60 %	≤50
Liberase TM Research Grade	10 mg	3.5 – 6.0	≥60 %	≤50
	100 mg	35 – 60		
Liberase TH Research Grade	10 mg	3.7 – 6.0	≥60 %	≤50
	100 mg	36 – 60		

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

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

The actual values determined for each lot are available in the respective Certificates of Analysis. They can be retrieved from the Roche Applied Science Homepage (https://www.roche-applied-science.com/techresources).

In the following, the measurement of collagenase I and collagenase II integrity by HPLC is described. Almost no fragmentation of both collagenase enzymes can be detected in Liberase TM Research grade blends, proving the purity of this enzyme mixture (Figure 1).

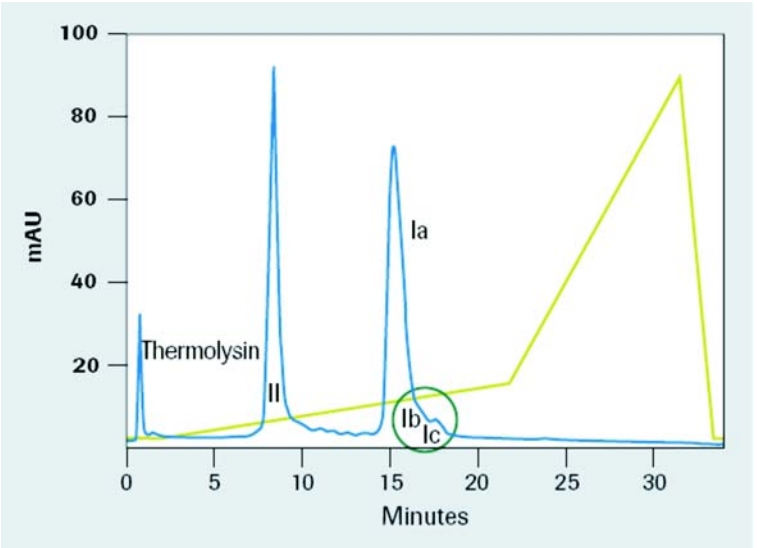

Fig. 1: HPLC analysis of Liberase TM Research Grade. Collagenase Ia represents the functionally active part of Collagenase I, whereas the degradation products are represented by Collagenase Ib and Ic peaks as indicated by the circle (II: Collagenase II; Ia, Ib, Ic: Collagenase Ia, Ib, Ic, respectively).

5. Supplementary Information

5.1 Conventions

5.1.1 Text Conventions

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

Text Convention	Usage
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.

5.1.2 Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- Specification range for "Collagenase content (mg/vial)" changed for Liberase DH Research Grade 10 mg and 100 mg in chapter 4.3. Quality control.
- Editorial changes

5.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com.

Product	Pack Size	Cat. No.
Liberase DL Research Grade	10 mg	05 401 160 001
Liberase DL Research Grade	100 mg	05 466 202 001
Liberase TL Research Grade	10 mg	05 401 020 001
Liberase DH Research Grade	10 mg	05 401 054 001
Liberase DH Research Grade	100 mg	05 401 089 001
Liberase TM Research Grade	10 mg	05 401 119 001
Liberase TM Research Grade	100 mg	05 401 127 001
Liberase TH Research Grade	10 mg	05 401 135 001
Liberase TH Research Grade	100 mg	05 401 151 001
Liberase Selection Kit Research Grade	5 mg each	05 401 046 001

5.4 Trademarks and Disclaimer

Regulatory Disclaimer

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

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- Certificates of Analysis
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- Lab FAQS: Protocols and references for life science research

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