

Saltonase® ELISA Kit Handbook

For the quantification of Saltonase content in cellular lysate or eluate from protein purification processes

Table of Contents

| Kit Contents | 3 |
|--|----------------------|
| Shipping and Storage | 4 |
| Intended Use | 5 |
| Safety Information | 6 |
| Quality Control | 7 |
| Introduction Principle of the assay | 8 |
| Equipment and Reagents to Be Supplied by User | 9 |
| Important Notes Preparing reagents Preparing the standard Preparing the sample | 10 |
| Important points before starting | 12 12 12 13 |
| Troubleshooting Guide | 14 |
| | 15 |
| | 16 |

Kit Contents

Saltonase ELISA Kit Catalog number No. of reactions

EN32-001 5 × 96

| | | | | Storage temperature |
|--|--|-----------|--------|---------------------|
| Part | Description | Format | Volume | (°C) |
| Box 1* | | | | |
| Capture Plate | 96-well strip microplate (12 strips of 8 wells) | 5 plates | - | 2–8 |
| Detection A | Vial of biotin labeled antibody | 1 vial | 550 µL | 2–8 |
| Detection B | Vial of streptavidin conjugated to HRP | 1 vial | 550 pL | 2–8 |
| Dilution Buffer | 25 mL per bottle diluent (including preservative) used to dilute the protein standard and detection reagents A/B | 2 bottles | 25 mL | 2–8 |
| 20x Wash Buffer Concentrate [†] | 25 mL per bottle of a 20-fold concentrated solution of buffered surfactant | 1 bottle | 110 mL | 2–8 |
| Color Reagent | 50 mL per bottle of TMB (tetramethylbenzidine) | 1 bottle | 50 mL | 2–8 |
| Stop Solution | 25 mL per bottle | 1 bottle | 25 mL | 2–8 |
| Plate Sealer | Adhesive strips | 20 strips | - | 2–8 |
| Box 2 [§] | | | | |
| Saltonase standard | Vial of Saltonase MBG (100 µg/mL) | 1 vial | 1 mL | -30 to -15 |

^{*} Shipped at 4°C.

[†] Dilute 1:20 with deionized water before use.

[§] Shipped at -20°C.

Shipping and Storage

Saltonase ELISA Kit is shipped in 2 boxes. When you receive the kit, promptly store reagents at the indicated temperatures.

- Box 1 is shipped at 4°C under controlled temperature conditions. Upon receipt, all components in Box 1 should be stored at 2–8°C.
- Box 2 is shipped at -20°C. Upon receipt, the vial containing Saltonase MBG in Box 2 should be stored at -30°C to -15°C.

Under these conditions, the components are stable until the expiration date. The expiration date for the product is provided on the label and will vary based on the date of manufacture of the kit.

Intended Use

Saltonase ELISA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Enzyme Stop Solution is an acidic solution that may cause severe skin burns and eye damage. Wear eyes, hands, face, and clothing protection when using this material.

Saltonase Detection Antibody A, Dilution Buffer, and Conjugate Detection Antibody B may cause allergic reaction. Wear eyes, hands, face, and clothing protection when handling these materials.

Detection A and Dilution Buffer can cause an immediate skin, eye, or respiratory tract irritant. Use protective gloves, protective clothing, eyes protection, and face protection.

Enzyme Substrate Solution (Color Reagent) can cause skin and eye irritation, and is suspected of causing cancer. Wear eyes, hands, face, and clothing protection when using this material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Saltonase ELISA Kit is tested against predetermined specifications to ensure consistent product quality. Saltonase ELISA Kit meets ISO 13485 requirements.

Introduction

Saltonase ELISA Kit (5 \times 96) is for the quantification of Saltonase content in cellular lysate or eluate from protein purification processes.

Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Saltonase has been pre-coated onto a microplate. Standards or samples are pipetteted into the wells and any Saltonase present is bound by the immobilized antibody. After washing away any unbound reagent, a biotin-labeled Saltonase antibody (Detection A) is added to the wells. After washing away any unbound reagent, Streptavidin-Poly-HRP (Detection B) is added to the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Saltonase bound in the initial step. The color development is stopped and the intensity of the color is measured using a plate reader.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

Make sure that you have the following user-supplied consumables and equipment before starting the protocol. These items supplement the components provided in the kit.

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm
- · Pipettes and pipette tips
- · Deionized or distilled water
- 500 mL graduated cylinder
- Squirt bottle, multichannel pipette, or automated microplate washer
- Test tubes for dilution of standards

Important Notes

Preparing reagents

Equilibrate all reagents to room temperature (15-25°C) before use.

20-fold Wash Buffer Concentrate

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of diluted Wash Buffer.

Detection A/B

Shake and mix before use, then briefly centrifuge to ensure the whole volume is at the bottom of the tube. Dilute the Detection A and B reagents 1:100 to the working concentration with Dilution Buffer.

Preparing the standard

Thaw the Saltonase standard (100 μ g/mL) at room temperature and dilute stepwise with the provided Dilution Buffer to reach a final concentration of 100 ng/mL. For example:

- 1. 20 µL Saltonase standard + 380 µL Dilution Buffer (1:20 dilution)
- 2. 20 µL of step 1 dilution + 380 µL Dilution Buffer (1:20 dilution)
- 3. 100 μL of step 2 dilution + 150 μL dilution buffer (1:2.5 dilution). Using the diluted standard (ST), prepare the standard curve by serial dilutions according to Table 1 on the facing page. Dilution Buffer is used to perform the serial dilutions. The resulting concentration of Saltonase in each sample is indicated in the following table.

Table 1. Serial dilution of the standard

| Sample | Preparation | Concentration (ng/mL) |
|-----------|---|-----------------------|
| Α | 16 μL diluted standard (ST) + 784 μL buffer | 2 |
| В | 400 μL sample A + 400 μL buffer | 1 |
| С | 400 μL sample B + 400 μL buffer | 0.5 |
| D | 400 μL sample C + 400 μL buffer | 0.25 |
| Е | 400 μL sample D + 400 μL buffer | 0.125 |
| F | 400 μL sample E + 400 μL buffer | 0.0625 |
| G | 400 μL sample F + 400 μL buffer | 0.03125 |
| H (blank) | 800 µL buffer | 0 |

Preparing the sample

It is recommended to dilute the sample in the provided Dilution Buffer to limit the influence of the sample matrix on the measurement. The tested assay range is from $31.25 \, pg/mL$ to $2000 \, pg/mL$.

Protocol

Important points before starting

- Equilibrate all reagents and samples to room temperature (15–25°C) before use.
- It is recommended that all standards and samples be assayed in at least duplicates.
- It is recommended that the required reagents and working standard are prepared immediately before use following the procedure described in the previous sections.

Assay procedure

- 1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
- 2. Add 100 μ L standard or samples to each well. Cover with the adhesive strip provided. Incubate for 1 h at room temperature without shaking.
- 3. Aspirate each well and wash, repeating the process 3 times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or auto-washer. Leave the wash solution in contact with the well for 30 s per wash.

Note: Complete removal of liquid at each step is essential for good assay performance.

- After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 4. Add 100 μ L of Detection A (working solution) to each well. Cover with a new adhesive strip. Incubate for 1 h at room temperature without shaking.
- 5. Repeat the aspiration/wash in step 3.

- 6. Add $100 \mu L$ of Detection B (working solution) to each well. Cover with a new adhesive strip. Incubate for $30 \min$ at room temperature without shaking.
- 7. Repeat the aspiration/wash in step 3.
- 8. Add 100 μL of Color Reagent to each well. Incubate for 10 min at room temperature. Protect from light.
- 9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color changes do not appear uniform, gently tap the plate to ensure thorough mixing.
- 10. Determine the optical density of each well within 10 min using a microplate reader set to 450 nm.

If wavelength correction is available, set it to 630 nm or 620 nm.

If wavelength correction is not available, subtract readings at 630 nm or 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation of results

Average the replicate readings for each standard and sample. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

To achieve the maximum accuracy, it is recommended to perform the measurements of the standard curve and the samples in triplicates.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, visit www.qiagen.com/FAQ/FAQList.aspx for Frequently Asked Questions at our Technical Support Center (for contact information, visit www.qiagen.com).

Comments and suggestions

| Poor precision | |
|--|--|
| a) Wells are not washed or aspirated properly | Make sure that the wash apparatus works properly, and that the protocol is followed. |
| b) Bubbles in the wells | Gently tap the plate to disperse bubbles. |
| c) Wells are scratched with pipette tip or washing needles | Dispense and aspirate solution into and out of wells with caution. |
| d) Particulate matter found in the samples | Remove any particulate matter by centrifugation prior to working on the assay. $ \\$ |
| High background | |
| a) Plate is not washed properly | Make sure that the wash apparatus works properly, and that the protocol is followed. |
| b) Incorrect incubation times and/or temperatures | The OD value increases gradually over time. Reduce the color development time. |
| Weak or no signal | |
| a) pipetteting errors | Make sure that the pipette is calibrated. |
| b) The working solution was not prepared immediately before use | The working solution should be prepared immediately before use and should not be stored. |
| c) Volumetric errors | Repeat assay with the volumes stated in this handbook. |
| d) The plate is not incubated for the proper time or temperature | Follow this handbook to repeat processing the assay. |

Ordering Information

| Product | Contents | Cat. no. |
|------------------------------|--|----------------------------|
| Saltonase ELISA Kit (5 x 96) | 60 pre-coated 8-well strips, providing a total of 480 measurements | EN32-001 |
| Related products | | |
| Saltonase MBG | Saltonase MBG at ≥250 U/µL | EN32-050, EN32-250, EN32-B |
| Saltonase GMP-grade | Saltonase GMP-grade at ≥250 U/µL | EN32-B10, EN32-B50 |

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

| Date | Description |
|---------|-----------------|
| 10/2025 | Initial release |

Limited License Agreement for Saltonase ELISA Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

- 1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the panel only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
- 2. Other than expressly stated licenses, QIAGEN makes no warranty that this panel and/or its use(s) do not infringe the rights of third-parties.
- 3. This panel and its components are licensed for one-time use and may not be reused, refurbished, or resold.
- 4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
- 5. The purchaser and user of the panel agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the panel and/or its components.

For updated license terms, see www.qiagen.com.

Trademarks: QIAGEN®, Sample to Insight®, Saltonase® (QIAGEN Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

10/2025 HB-3747-001 © 2025 QIAGEN, all rights reserved.

This page intentionally left blank

This page intentionally left blank

This page intentionally left blank