For quality control/manufacturing of IVD/
medical devices/pharmaceutical products only.

Residual Protein Trypsin Kit

Version 22

Content version: August 2022

Cat. No. 07 568 975 001 1 kit

Kit for 96 reactions

Store the kit at +2 to +8°C.

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Table of Contents

1. What this Product Does

Number of Tests The kit is designed for 96 reactions.

Kit contents

Vial/ Cap	Label	Function	Contents
1 white	Incubation Buffer	For preparation of the Biotin DIG Working Solution	▪ 100 ml ▪ Ready-to-use
2 blue	Conjugate Buffer	For preparation of the Anti-Dig POD Reagent, Vial 6	▪ 100 ml ▪ Ready-to-use
3 yellow	Biotin Conjugate	Biotin labeled for target capture	▪ 0.75 ml ▪ 20-fold stock solution ▪ Polyclonal anti-trypsin sheep antibody against recombinant trypsin
4 purple	DIG conjugate	Digoxigenin labeled for target marking	▪ 0.75 ml ▪ 20-fold stock solution ▪ Polyclonal anti-trypsin sheep antibody against recombinant trypsin
5 red	Wash Buffer	For washing steps	▪ 100 ml ▪ 10-fold stock solution
6 red	Anti-DIG POD Reagent	Peroxidase conjugated to anti-DIG antibody	▪ Lyophilizate
7 black	Detection Substrate (TMB)	For color development and detection	▪ 15 ml ▪ Ready-to-use
8 colorless	Stop Solution	For stopping the color development	▪ 15 ml ▪ Ready-to-use
9 sand	Standard A	For calibration of the assay	▪ Lyophilizate ▪ Trypsin free
10 beige	Standard B	For calibration of the assay	▪ Lyophilizate ▪ Recombinant trypsin
11 mustard	Standard C	For calibration of the assay	▪ Lyophilizate ▪ Recombinant trypsin

Vial/ Cap	Label	Function	Contents
12 olive	Standard D	For calibration of the assay	▪ Lyophilizate ▪ Recombinant trypsin
13 caramel	Standard E	For calibration of the assay	▪ Lyophilizate ▪ Recombinant trypsin
14 rose-wood	Standard F	For calibration of the assay	▪ Lyophilizate ▪ Recombinant trypsin
15 white	Control X	Positive control	▪ Recombinant trypsin ▪ Lyophilizate
16 green	Control Y	Positive control	▪ Recombinant trypsin ▪ Lyophilizate
17 MWP	Coated Multi-well Plate	Target capture	▪ Streptavidin- coated multi-well plate ▪ 12 strips in frame ▪ Ready-to-use
18 Film	MWP-sealing film	Sealing MWP during incubations	▪ 10 pieces of self-adhesive film

Storage and Stability

- Store kit at +2 to +8°C.
- This kit is stable until the expiration date printed on the label when stored unopened at +2 to +8°C.

Specificity and Sensitivity

This ELISA kit detects Trypsin*, recombinant from porcine pancreas, expressed in *Pichia pastoris*, available from Roche. See Chapter 7.3, Ordering Information for more details. Trypsin from other suppliers may show a different reactivity with regard to sensitivity and accuracy; therefore the compatibility of the kit calibration to the individual trypsin product must be verified. This ELISA kit is not suitable for measurement of non-porcine trypsin.

Sensitivity:	≤0.5 ng/ml
Measuring range:	0.5 ng/ml up to approximately 50 ng/ml. (Exact upper limit depends on the lot-specific concentration of Standard F. See lot-specific information in Chapter 3.)
Intra-assay precision:	≤10% (typically ≤ 5%)
Inter-assay precision:	≤15% (typically ≤10%)

Additional Equipment and Reagents Required

- Polypropylene tubes for preparation of antibody reagents
- 1 liter bottle for wash buffer preparation
- Micropipettes
- Centrifuge
- Multi-well plate shaker
- Roller mixer
- ELISA reader
- Multi-well plate washer

Application

This kit is intended for quantitative detection of residual trypsin in manufacturing processes of biopharmaceutical products.

Assay Time

- Incubation time: 1 h 45 min.
- Total assay time: approximately 2 to 2.5 hours

2. How to Use this Product

2.1 Before You Begin

Preparation of Sample Material

This kit is intended for use with the following types of sample material:

- Aqueous buffer solutions from biotechnology processes.
- Cell culture supernatant

⚠ If cell culture supernatant is used as the sample material, first test a sample of fresh, unused media for a potential background absorbance as some media may contain residual trypsin from production procedures. When testing fresh, unused media, ensure that all supplements required for the culture conditions have been added to the media.

Preparation of Positive and Negative Controls

⚠ Always ensure that positive and negative controls are tested on each plate together with samples.

Positive controls: Control X and Y are positive controls and are provided with the kit. For preparation of the positive controls, see "Preparation of Working Solutions".

Negative control: Analyte-free matrix of your sample material, such as buffer or cell culture medium.

Preparation of Working Solutions

⚠ Use double-distilled or deionized water of equivalent quality, for reconstitution of the lyophilizates. Ensure that the lyophilizates are carefully reconstituted to avoid trypsin contamination of the environment. Trypsin contamination of the workspace may potentially contaminate samples to be tested.

A roller mixer may be used to effectively dissolve lyophilizates.

In addition to the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content/ Vial	Preparation	Function	Storage and Stability
1 Standards, Vials 9 to 14	Add 500 µl of water to each of the vials and mix thoroughly for 30 min to completely dissolve the lyophilizate.	Calibration of the assay	At +2 to +8°C for 4 weeks.
2 Controls, Vials 15, 16	Add 500 µl of water to each of the vials and mix thoroughly for 30 min to completely dissolve the lyophilizate.	Positive controls	At +2 to +8°C for 4 weeks.

Content/ Vial	Preparation	Function	Storage and Stability
3 Wash Buffer Vial 5	Dilute the entire contents of the bottle (100 ml) with 900 ml water and mix thoroughly.	Washing steps	At +2 to +8°C for 4 weeks.
4 Biotin Conjugate, Vial 3	Dilute the appropriate volume 1:10 with Incubation Buffer from Vial 1. For a complete 96-well plate, prepare 500 µl of Biotin Conjugate stock solution + 4.5 ml of Incubation Buffer. If using only a part of the 96 wells, then prepare a proportionally reduced volume of this working solution.	Target capture	At +2 to +8°C for 4 weeks.
5 DIG Conjugate, Vial 4	Dilute the appropriate volume 1:10 with Incubation Buffer from Vial 1. For a complete 96-well plate, prepare 500 µl of DIG Conjugate stock solution + 4.5 ml of Incubation Buffer. If using only a part of the 96 wells, then prepare a proportionally reduced volume of this working solution.	Target marking	At +2 to +8°C for 4 weeks.
6 Anti-DIG POD Reagent, Vial 6	1. Add 500 µl water to the vial and mix thoroughly for 30 min to completely dissolve the lyophilizate. The concentration of the reconstituted solution is 4 U/ml. 2. Dilute the 4 U/ml solution with Conjugate Buffer (Vial 2) in 2 steps to achieve a working concentration of 15 mU/ml, as follows: ▪ Step A: 50 µl POD (4 U/ml) + 450 µl Conjugate Buffer, ▪ Step B: 375 µl product from Step A + 9.625 ml Conjugate Buffer	Detection	At +2 to +8°C for 4 weeks.

Content/ Vial	Preparation	Function	Storage and Stability
7 Biotin DIG Working Solution	Combine equal volumes of the prepared working solutions: Biotin Conjugate and DIG Conjugate (from Steps 4 and 5 above). Mix thoroughly, for example, using a roller mixer for 10 min	ELISA Step 6	Use within 30 min after preparation.

- The controls supplied with the kit are ready to use once reconstituted. Do not further dilute the Positive Controls X or Y (Vials 15 and 16) provided with the kit; prepare as described in the Chapter "Preparation of Working Solutions".
- ⚠ Always prepare the Biotin DIG Working Solution just before use, and use the solution within 30 minutes after preparation. Do not store the prepared mixture for a longer time!

2.2 Procedure

Sample Preparation

If cell-free aqueous solutions are used as samples, no sample preparation is required. For testing of cell culture samples, the samples must be centrifuged and the supernatants can be used for trypsin determination.

If there are samples with an expected trypsin concentration of approximately >50 ng/ml, or the photometric absorbance of a sample measurement is higher than the measured absorbance of the highest Standard F, then dilute the sample (*e.g.,* 1:20 and 1:100) with Incubation Buffer from Vial 1 and test the diluted sample.

ELISA Procedure

- The ELISA was developed and evaluated using 10 µl sample and 90 µl immunoreagent per well of the Multi-well Plate. Roche recommends not changing these volumes.
- Roche recommends performing measurements of at least duplicates of each sample, standards, and controls.
- Include the standards provided with the kit on the same plate in each run to generate quantitative results. For quality control of the correct function of all components of the assay, measure the positive controls provided with the kit (Controls X and Y; Vials 15 and 16) together with the samples on the same plate in each run. An analyte-free sample of your sample matrix should also be measured on the same plate as a negative control.
- Working temperature for all of the procedures described below is +15 to +25°C.

Step	Action
❶	Transfer 10 µl per well of the following materials in duplicate to 2 wells each on the coated Multi-well Plate: ▪ Standards A to F (Vials 9 to 14) ▪ Positive Controls X and Y (Vials 15 and 16) ▪ Negative control of your sample matrix (cell culture medium or aqueous buffer solution) ▪ Samples (centrifuged cell culture supernatants or aqueous solutions from your biotechnological process step)
❷	Add 90 µl of the fresh Biotin DIG Working Solution to each well.
❸	Seal the Multi-well Plate with an MWP-sealing film.
❹	Incubate the sealed plate on a multi-well plate shaker at 300 rpm and +15 to +25°C for 1 h.
❺	Remove the film carefully and wash the Multi-well Plate 3 times with 300 µl per well of the diluted 1x Wash Buffer using a multi-well plate washer.
❻	Pipet 100 µl of the diluted working solution of the Anti-Dig POD Reagent (15 mU/ml) into each well.
❼	Seal the Multi-well Plate with an MWP-sealing film.
❽	Incubate the sealed plate on a multi-well plate shaker at 300 rpm and +15 to +25°C for 30 min.
❾	Remove the film carefully and wash the Multi-well Plate 3 times with 300 µl per well of the diluted 1x Wash Buffer using a multi-well plate washer.
❿	Pipet 100 µl Detection Substrate (TMB) (Vial 7) into each well.
⓫	Seal the Multi-well Plate with an MWP-sealing film.
⓬	Incubate the sealed plate on a multi-well plate shaker at 300 rpm and +15 to +25°C for 15 min.
⓭	Remove the film carefully and pipet 50 µl of Stop Solution (Vial 8) into each well.
⓮	Seal the Multi-well Plate with an MWP-sealing film.
⓯	Incubate the sealed plate on a multi-well plate shaker with 300 rpm for 1 min at +15 to +25°C.
⓰	Place the plate in a multi-well plate reader and measure at 450 nm, reference wavelength 620 nm.

Calculation of Concentrations

The calculation is described in the following table:

Step	Action
❶	Calculate the mean of the absorbance values from the replicate measurements. The mean values are used for the calculations in the following steps.
❷	If the background absorbance value of the negative control is higher than the absorbance value of Standard A, the absorbance values of the samples must be corrected. ▪ Subtract the absorbance value of Standard A from the absorbance value of the negative control. ▪ Subsequently, subtract the corrected negative control absorbance value from the absorbance values of the samples. ▪ Use the obtained absorbance values to calculate the trypsin concentrations of the samples.
❸	Generate a calibration curve using the concentrations and absorbances of Standards A to F. Use the information from the lot-specific standard concentrations in the value table in Chapter 3 for the concentrations. Use a curve fitting software with a 4 parameter non-linear fit model to calculate the standard curve using the results of Standards A to F.
❹	Use the absorbance values of the controls, as well as the corrected absorbance values of the samples from Step 2, above, to determine the concentrations based on the standard curve created in Step 3.

2.3 Checks and Controls

Quality control of the test run

Check the recovery of the controls. The determined control concentrations should be within ± 25% of the lot-specific target concentration (see Chapter 3 for lot-specific concentrations of Controls X and Y). If the results of the control concentrations are not within ± 25% of the lot-specific target concentration, the sample results may be invalid.

Concentration out of range

If the mean absorbance of a sample is higher than the absorbance of the highest Standard F, then the sample concentration is higher than the concentration of Standard F. An exact concentration value for a sample cannot be calculated when the sample has an absorbance value that is higher than the absorbance of the Standard F. To determine the concentration of such a sample, repeat the test with a diluted sample. Dilute the sample with Incubation Buffer (Vial 1) at, for example, 1:20 and 1:100. Take the dilution factor into consideration when calculating the concentration with the absorbance values of the diluted sample.

3. Lot-Specific Data

The table below provides the lot-specific concentrations of the standards and controls. Use these values to generate and verify the standard curve for each test run.

Cat. No.: **07 568 975 001**

Lot. No.: **67309700**

Expiration date: **Sep 2023**

Vial/Cap	Label	Concentration
9 sand	Standard A	0.0 ng/ml
10 beige	Standard B	1.6 ng/ml
11 mustard	Standard C	4.6 ng/ml
12 olive	Standard D	13.3 ng/ml
13 caramel	Standard E	25.8 ng/ml
14 rosewood	Standard F	51.5 ng/ml
15 white	Control X	6.3 ng/ml
16 green	Control Y	26.6 ng/ml

4. Typical Results

Performance

Performance data provided below represent typical data. Individual results may vary depending on the sample matrix and the test run. The calibration curve shown below is only an example of typical results. The appropriate calibration values need to be generated for each run, with the standards placed on the same plate as the samples within a run.

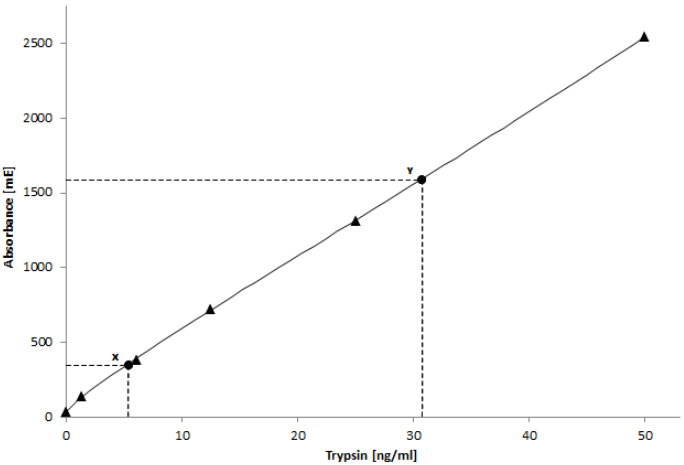

Fig. 1: Example of a standard curve using a 4-parametric non-linear fit model. Actual absorbance may vary according to the individual test run.

Examples of sample absorbance and calculation of concentration values for the standard curve shown in Figure 1:

Control X = 362 mE = 5.5 ng/ml
Control Y = 1,568 mE = 30.2 ng/ml

5. Troubleshooting

Problem	Possible Cause	Recommendation
Absorbance of samples too low	Insufficient TMB reaction	Increase the incubation time with the Detection Substrate (TMB) for color development (Step 12). The incubation time can be increased until the wells with the higher concentrated standards show a clearly observable blue color, up to 40 min.
	POD conjugate has a reduced enzymatic activity.	Use freshly prepared POD conjugate.
	Low concentration after dilution of highly concentrated samples	Reduce dilution factor for highly concentrated samples.
Absorbance of samples too high	Concentration exceeds measuring range.	Dilute samples to ensure that they are within the specified detection range.
	Background of sample matrix.	Check analyte-free sample matrix for background absorbance value. Correct sample values for background absorbance of matrix.
	Contamination of samples with trypsin from lab workspace.	Check absorbance values of Standard A and negative control. If the absorbance values of Standard A and the negative control are significantly increased, repeat the experiment in a different workspace using a new kit. Be careful when reconstituting lyophilizates. Thoroughly clean contaminated workspace.
Absorbance of negative control too high	Background of sample matrix.	Check for potential residual trypsin in the analyte-free matrix using a different lot of the matrix.
	Contamination of samples with trypsin from lab workspace.	Check absorbance values of Standard A and negative control. If the absorbance values of Standard A and the negative control are significantly increased, repeat the experiment in a different workspace using a new kit. Be careful when reconstituting lyophilizates. Thoroughly clean contaminated workspace.

Problem	Possible Cause	Recommendation
Absorbance of the background too high	Substrate shows color development without enzymatic activity.	Use a newly opened vial of Detection Substrate (TMB).
	Contamination of samples with trypsin from lab workspace.	Check absorbance values of Standard A and negative control. If the absorbance values of Standard A and the negative control are significantly increased, repeat experiment in a different workspace using a new kit. Be careful when reconstituting lyophilizates. Thoroughly clean contaminated workspace.
Variations too high	Insufficient mixing of sample and incubation mix.	Ensure mixing on the multi-well plate mixer is done with 300 rpm.
	Residual buffer after washing	Check performance of multi-well plate washer. Wells should not contain residual buffer after washing. Try tapping the Multi-well Plate on an absorbent paper towel to remove traces of Wash Buffer.
	Sample matrix difficult to pipet due to viscosity or composition.	Carefully pipet samples without droplets on outside of tip. Avoid high aspiration or ejection velocity when pipetting the samples.
	Precision of micropipettes	Check the precision of the micropipettes.
	Multi-well plate washer not washing correctly.	Check the multi-well plate washer for tip blockage or salt crystallization, which may affect the evenness and effectiveness of the washing steps.

6. Additional Information on this Product

6.1 How this Product Works

The assay is based on a quantitative sandwich enzyme linked immunoassay principle using sheep polyclonal antibodies directed against recombinant porcine trypsin from Roche. As polyclonal antibodies detect a multitude of epitopes, the assay allows for the sensitive detection of full length as well as fragmented trypsin.

6.2 Test Principle

Step	Action
❶	The sample is placed into a streptavidin-coated multi-well plate.
❷	A mixture of biotinylated and digoxigenylated anti-trypsin antibodies are added and incubated with the sample. During the incubation period, the antibodies form a sandwich with trypsin that is bound to the streptavidin-coated multi-well plate.
❸	Unbound components are removed by a washing step.
❹	Anti-DIG POD conjugate is added. It binds to the digoxigenylated anti-trypsin antibody.
❺	Unbound components are removed by a washing step.
❻	Addition of the color substrate TMB. POD retained in the immuno sandwich converts TMB to a detectable colored dye.
❼	Addition of the acidic Stop Solution. Photometric measurement of generated dye from the converted TMB substrate is used for calculation of the trypsin concentration.

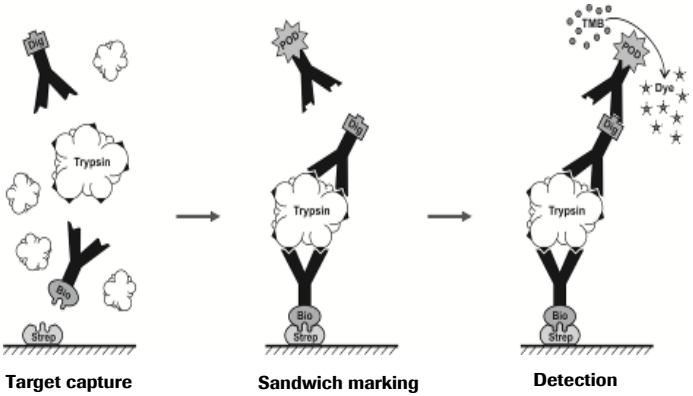

Fig. 2: Principle of the Residual Protein Trypsin ELISA.

6.3 Quality Control

A quantitative, functional assay is performed to assess background signal, and the lot-specific concentrations of Standards A to F and Controls X and Y.

7. Supplementary Information

7.1 Conventions

Text Conventions To make information consistent and easy to understand, the following text conventions are used throughout this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷ *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk*	Denotes a product available from Roche Diagnostics.

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.

7.2 Changes to Previous Version

- New lot-specific data provided in Chapter 3.

7.3 Ordering Information

Roche offers a large selection of reagents and systems for systems for quality control and manufacturing processes. For a complete overview of related products and manuals, please visit and bookmark our homepage www.custombiotech.roche.com

Product	Pack Size	Cat. No.
Trypsin, recombinant, from porcine pancreas, expressed in *Pichia pastoris*	3.5 MU	03 358 658 103
Trypsin, recombinant, from porcine pancreas, expressed in *Pichia pastoris*	1 g = 0.23 MU	06 369 880 103

7.4 Trademarks

7.5 Regulatory Disclaimer

For quality control/manufacturing of IVD/
medical devices/pharmaceutical products only.

7.6 Contact and Support

For additional documentation such as certificates and safety data sheets, please visit documentation.roche.com.

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

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