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Residual Protein Trypsin Kit

Kit for determination of trypsin

Version: 37

Content Version: August 2024

Cat. No. 07 568 975 001 1 kit
96 reactions

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function/Description	Content
1	white	Residual Protein Trypsin Kit, Incubation Buffer	▪ Ready-to-use solution. ▪ For preparation of the Biotin DIG working solution.	1 bottle, 100 mL
2	blue	Residual Protein Trypsin Kit, Conjugate Buffer	▪ Ready-to-use solution. ▪ For preparation of the Anti-DIG POD Reagent, Bottle 6.	1 bottle, 100 mL
3	yellow	Residual Protein Trypsin Kit, Biotin Conjugate	▪ Biotin labeled for target capture. ▪ Polyclonal anti-trypsin sheep antibody against recombinant trypsin. ▪ 20-fold stock solution	1 vial, 0.75 mL
4	purple	Residual Protein Trypsin Kit, DIG Conjugate	▪ Digoxigenin labeled for target marking. ▪ Polyclonal anti-trypsin sheep antibody against recombinant trypsin. ▪ 20-fold stock solution	1 vial, 0.75 mL
5	red	Residual Protein Trypsin Kit, Wash Buffer	▪ For washing steps. ▪ 10-fold stock solution	1 bottle, 100 mL
6	red	Residual Protein Trypsin Kit, Anti-DIG POD Reagent	▪ Peroxidase conjugated to anti-DIG antibody. ▪ Lyophilized	1 bottle
7	black	Residual Protein Trypsin Kit, Detection Substrate (TMB)	▪ For color development and detection. ▪ Ready-to-use solution.	1 bottle, 15 mL
8	colorless	Residual Protein Trypsin Kit, Stop Solution	▪ For stopping the color development. ▪ Ready-to-use solution.	1 bottle, 15 mL
9	sand	Residual Protein Trypsin Kit, Standard A	▪ For calibration of the assay. ▪ Lyophilized ▪ Trypsin free	1 bottle
10	beige	Residual Protein Trypsin Kit, Standard B	▪ For calibration of the assay. ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
11	mustard	Residual Protein Trypsin Kit, Standard C	▪ For calibration of the assay. ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
12	olive	Residual Protein Trypsin Kit, Standard D	▪ For calibration of the assay ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
13	caramel	Residual Protein Trypsin Kit, Standard E	▪ For calibration of the assay ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
14	rosewood	Residual Protein Trypsin Kit, Standard F	▪ For calibration of the assay ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
15	white	Residual Protein Trypsin Kit, Control X	▪ Positive control ▪ Recombinant trypsin ▪ Lyophilized	1 bottle
16	green	Residual Protein Trypsin Kit, Control Y	▪ Positive control ▪ Recombinant trypsin ▪ Lyophilized	1 bottle

17	–	Residual Protein Trypsin Kit, Coated Multiwell Plate	▪ Target capture ▪ Streptavidin-coated multiwell plate ▪ Ready-to-use.	12 strips in frame
18	–	Residual Protein Trypsin Kit, Multiwell Sealing Film	▪ Sealing MWP during incubations.	10 pieces of self-adhesive film

1.2. Storage and Stability

Storage Conditions (Product)

The reagents are stable at +2 to +8°C until the expiry date printed on the label when stored unopened and kept free of contamination.

Vial / Bottle	Cap	Label	Storage
1	white	Incubation Buffer	Store at +2 to +8°C.
2	blue	Conjugate Buffer	
3	yellow	Biotin Conjugate	
4	purple	DIG Conjugate	
5	red	Wash Buffer	
6	red	Anti-DIG POD Reagent	
7	black	Detection Substrate (TMB)	
8	colorless	Stop Solution	
9	sand	Standard A	
10	beige	Standard B	
11	mustard	Standard C	
12	olive	Standard D	
13	caramel	Standard E	
14	rosewood	Standard F	
15	white	Control X	
16	green	Control Y	
17	–	Coated Multiwell Plate	
18	–	Multiwell Sealing Film	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

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

1.4. Preparation Time

Assay Time

- Incubation time: 1 hour 45 minutes.
- Total assay time: approximately 2 to 2.5 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

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.***

Control Reactions

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

Positive controls

Controls X and Y are positive controls and are provided with the kit. For preparation of the positive controls, see Section **Working Solution**.

Negative control

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

Safety Information

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.

Working Solution

⚠ ***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:

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
1	Standards A to F (Bottles 9 to 14)	Add 500 µL of double-distilled water to each of the bottles and mix thoroughly for 30 minutes to completely dissolve the lyophilizate.	Store at +2 to +8°C for 4 weeks.	Calibration of the assay
2	Controls X and Y (Bottles 15 and 16)	▪ Add 500 µL of double-distilled water to each of the bottles and mix thoroughly for 30 minutes to completely dissolve the lyophilizate. ▪ The controls supplied with the kit are ready to use once reconstituted. ⚠ ***Do not further dilute the Positive Controls X and Y.***	Store at +2 to +8°C for 4 weeks.	Positive controls
3	Wash Buffer (Bottle 5)	Dilute the entire contents of the bottle (100 mL) with 900 mL water and mix thoroughly.	Store at +2 to +8°C for 4 weeks.	Washing steps
4	Biotin Conjugate (Vial 3)	▪ Dilute the appropriate volume 1:10 with Incubation Buffer from Bottle 1. ▪ For a 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, prepare a proportionally reduced volume of this working solution.	Store at +2 to +8°C for 4 weeks.	Target capture
5	DIG Conjugate (Vial 4)	▪ Dilute the appropriate volume 1:10 with Incubation Buffer from Bottle 1. ▪ For a 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, prepare a proportionally reduced volume of this working solution.	Store at +2 to +8°C for 4 weeks.	Target marking

6	Anti-DIG POD Reagent (Bottle 6)	▪ Add 500 µL double-distilled water to the bottle and mix thoroughly for 30 minutes to completely dissolve the lyophilizate. The concentration of the reconstituted solution is 4 U/mL. ▪ Dilute the 4 U/mL solution with Conjugate Buffer (Bottle 2) in 2 steps to achieve a working concentration of 15 mU/mL, as follows: ▪ Step A: 50 µL Anti-DIG POD Solution (4 U/mL) + 450 µL Conjugate Buffer, ▪ Step B: 375 µL product from Step A + 9.625 mL Conjugate Buffer.	Store at +2 to +8°C for 4 weeks.	Detection
7	Biotin DIG Working Solution	Combine equal volumes of the prepared working solutions: Biotin Conjugate (Solution 4) and DIG Conjugate (Solution 5). Mix thoroughly, for example, using a roller mixer for 10 minutes. ⚠ ***Always prepare the Biotin DIG Working Solution just before use, and use the solution within 30 minutes after preparation.***	Use within 30 minutes after preparation.	ELISA Step 6

2.2. Protocols

Preparation of sample material

Culture supernatant

First test a sample of fresh, unused media for a potential background absorbance as some media may contain residual protease from production procedures. When testing fresh, unused media, ensure that all supplements required for the culture conditions have been added to the media.

Cell-free aqueous solution

No sample preparation is required.

Cell culture material

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, dilute the sample, for example, 1:20 and 1:100 with Incubation Buffer from Bottle 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 Multiwell Plate. Use the recommended volumes.

- Perform 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; Bottles 15 and 16) together with the samples on the same plate in each run and include a negative control.
- Working temperature for all of the procedures described below is +15 to +25°C.

1. Transfer 10 µL per well of the following materials in duplicate to 2 wells each on the coated Multiwell Plate:
   – Standards A to F (Bottles 9 to 14)
   – Positive Controls X and Y (Bottles 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).
2. Add 90 µL of the fresh Biotin DIG Working Solution to each well.
3. Seal the Multiwell Plate with an MWP-sealing film.
4. Incubate the sealed plate on a multiwell plate shaker at 300 rpm at +15 to +25°C for 1 hour.
5. Remove the film carefully and wash the Multiwell Plate 3 times with 300 µL per well of the diluted 1x Wash Buffer using a multiwell plate washer.
6. Pipette 100 µL of the diluted working solution of the Anti-DIG POD Reagent (15 mU/mL) into each well.
7. Seal the Multiwell Plate with an MWP-sealing film.
8. Incubate the sealed plate on a multiwell plate shaker at 300 rpm at +15 to +25°C for 30 minutes.
9. Remove the film carefully and wash the Multiwell Plate 3 times with 300 µL per well of the diluted 1x Wash Buffer using a multiwell plate washer.
10. Pipette 100 µL Detection Substrate (TMB) (Bottle 7) into each well.
11. Seal the Multiwell Plate with an MWP-sealing film.
12. Incubate the sealed plate on a multiwell plate shaker at 300 rpm at +15 to +25°C for 15 minutes.
13. Remove the film carefully and pipette 50 µL of Stop Solution (Bottle 8) into each well.
14. Seal the Multiwell Plate with an MWP-sealing film.
15. Incubate the sealed plate on a multi-well plate shaker with 300 rpm for 1 minute at +15 to +25°C.
16. Place the plate in a multiwell plate reader and measure at 450 nm, reference wavelength 620 nm.

Calculation of concentrations

1. Calculate the mean of the absorbance values from the replicate measurements. The mean values are used for the calculations in the following steps.

2. 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.

3. 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 Section **Lot-Specific Data**. 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.

4. Use the absorbance values of the controls, as well as the corrected absorbance values of the samples from Step 2 to determine the concentrations based on the standard curve created in Step 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 Section **Lot-Specific Data** for the 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 (Bottle 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.

2.3. Parameters

Detection range

0.5 ng/mL up to approximately 50 ng/mL.
Exact upper limit depends on the lot-specific concentration of Standard F. See Section **Lot-Specific data**.

Precision

Intra-assay precision: ≤10% (typically ≤5%)
Inter-assay precision: ≤15% (typically ≤10%)

Sensitivity

≤0.5 ng/mL
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.

3. Results

Typical results

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.

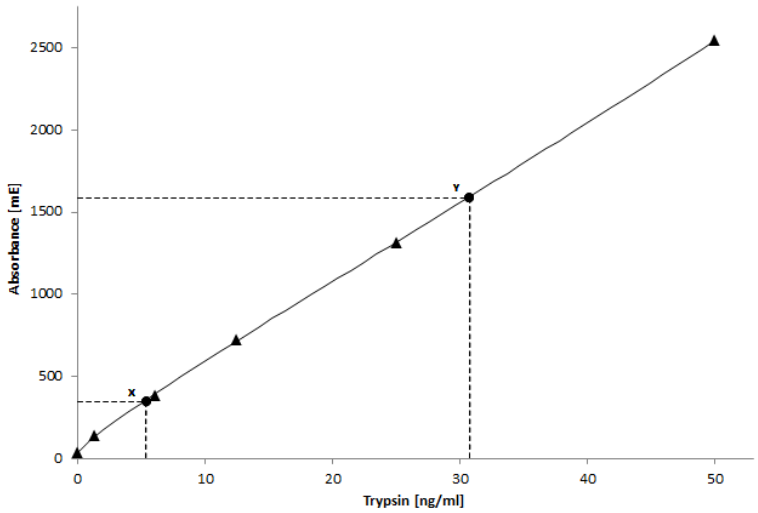

Fig. 1: Example of a standard curve using a 4-parametric non-linear fit model. Actual absorbance may vary according to the individual test run.Examples of sample absorbance and calculation of concentration values for the standard curve shown in Figure 1:
Control X = 362 mE = 5.5 ng/mL
Control Y = 1,568 mE = 30.2 ng/mL

4. Troubleshooting

Observation	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 minutes.
	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 the 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 the 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 the background too high.	Substrate shows color development without enzymatic activity.	Use a newly opened bottle of Detection Substrate (TMB).
	Contamination of samples with trypsin from lab workspace.	Check absorbance values of Standard A and the 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.***
Variations too high.	Insufficient mixing of sample and incubation mix.	Ensure mixing on the multiwell plate mixer is done with 300 rpm.
	Residual buffer after washing.	Check performance of multiwell plate washer. Wells should not contain residual buffer after washing. Try tapping the Multiwell Plate on an absorbent paper towel to remove traces of Wash Buffer.
	Sample matrix difficult to pipette due to viscosity or composition.	Carefully pipette 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.
	Multiwell plate washer not washing correctly.	Check the multiwell plate washer for tip blockage or salt crystallization, which may affect the evenness and effectiveness of the washing steps.

5. Additional Information on this Product

5.1. Test Principle

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

1. The sample is placed into a streptavidin-coated multiwell plate.

2. A mixture of biotinylated and digoxigenylated anti-trypsin antibodies is added and incubated with the sample.
   – During the incubation period, the antibodies form a sandwich with trypsin that is bound to the streptavidin-coated multiwell plate.

3. Unbound components are removed by a washing step.

4. Anti-DIG POD Conjugate is added.
   – It binds to the digoxigenylated anti-trypsin antibody.

5. Unbound components are removed by a washing step.

6. Addition of the color substrate TMB.
   – POD retained in the immunosandwich converts TMB to a detectable colored dye.

7. Addition of the acidic Stop Solution.
   – Photometric measurement of generated dye from the converted TMB substrate is used for calculation of the trypsin concentration.

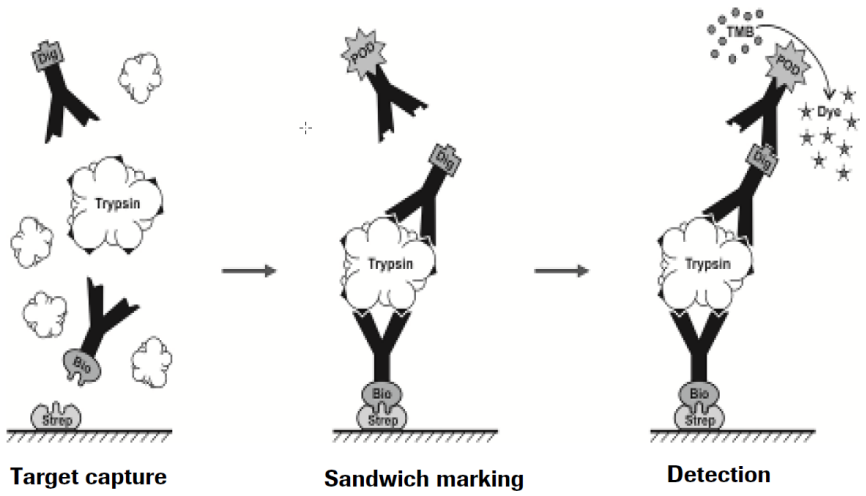

Fig. 2: Principle of the Residual Protein Trypsin ELISA.

5.2. 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.

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

New lot-specific data added.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Trypsin, recombinant	PC (1g = 0.23 MU)	06 369 880 103
	3.5 MU	03 358 658 103

6.4. Trademarks

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

6.5. License Disclaimer

Consult product detail pages at **custombiotech.roche.com** for patent license limitations, if available.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. Contact and Support

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

Your Roche CustomBiotech Customer Service:

Europe, Middle East, Africa and Latin America
Roche Diagnostics Deutschland GmbH
Phone +49 621 759 8580
Fax +49 621 759 6385
mannheim.custombiotech@roche.com

United States
Roche Diagnostics Corporation
Phone +1 800 428 5433 (toll free)
Fax +1 317 521 4065
custombiotech.ussales@roche.com

Canada
Roche Diagnostics
Phone +1 450 686 7050
Fax +1 450 686 7012
custombiotech.can@roche.com

Japan
Roche Diagnostics K.K.
Phone +81 3 6634 1046
Fax +81 3 5479 0585
japan.custombiotech@roche.com

Asia Pacific
Roche Diagnostics Asia Pacific Pte. Ltd.
Phone +65 6371 6638
Fax +65 6371 6601
apac.custombiotech@roche.com

0824.07668872001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany

7. 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.

Residual Protein Trypsin Kit	
REF	**07 568 975 001**
	37
LOT	**83878900**
	Aug 2025

Vial / Cap	Label	concentration (ng/mL)
9 sand	Standard A	0.0
10 beige	Standard B	2.0
11 mustard	Standard C	6.2
12 olive	Standard D	14.9
13 caramel	Standard E	29.2
14 rosewood	Standard F	56.7
15 white	Control X	7.2
16 green	Control Y	27.7