NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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Porcine Trypsin ELISA Kit
User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1402422
Version: A/0
For Research Use Only

Biofargo, Inc.

■ Product Name

Porcine Trypsin ELISA Kit

■ Package

96 tests/Kit

■ Intended Use

This kit is intended for determining the presence of porcine trypsin in the biological product during downstream purification process. The kit is suitable for detecting residual recombinant porcine trypsin and trypsin derived from porcine pancreas that are commonly on the market.

The kit is for RESEARCH USE ONLY and is not intended for clinical use.

■ Product Description

Porcine trypsin is a serine protease and cleaves peptides on the C-terminal of lysine and arginine residues, which can be used for the recombinant insulin production, the activation of viral particles, and dissociation a variety of adherent cells during cell culture.

This kit is based on the solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual trypsin in the samples. A sheep polyclonal antibody specific to trypsin was employed in the assay to capture any remaining trypsin in the samples. Both Calibration standards and test samples were added to the microtiter plate pre-coated with capture antibody. After washing out unbound proteins, the Detection Antibody and biotin conjugated anti-Trypsin antibody were added into the well to form the complexe. Following incubation and washing, the horseradish peroxidase conjugated Streptavidin(Streptavidin-HRP) is added. After another incubation and washing step, TMB (3,3',5,5' -tetramethylbenzidine) substrate was added into reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue product (maximum absorption peak at 655 nm). Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the Trypsin concentration in the calibration standards and the samples. The concentration of Trypsin in the samples can be calculated using a dose-response curve.

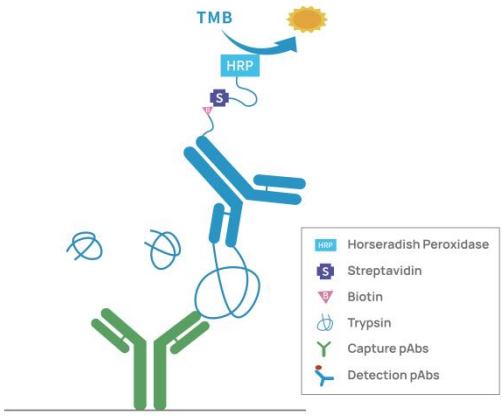


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
Trypsin Calibration Standard	PNB006	2 bottles	Lyophilized powder. Please refer to the details on the label of the tube.
Anti-Trypsin Microtiter Strips	PNA006	8 well × 12 strips	Strips pre-coated with sheep anti-Trypsin polyclonal antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC003	1 × 240μL	Only used for dissolving Trypsin Calibration Standard. Avoid direct contact with eyes, skin, and clothing.
Wash Buffer Concentrate (10×)	PNF001	3 × 25 mL	Easy to be crystallized at low temperature, please incubate at 37℃ in water bath before use. Dilute 10 times with freshly prepared ultra-pure water to obtain 1 × Wash Buffer for plate washing and dilution of Calibration Standard, Anti-Trypsin:Biotin (100×), Streptavidin-HRP (100×） and the samples.
Anti-Trypsin:Biotin (100×)	PNG006	1 × 140 μL	Sheep polyclonal antibody conjugated to biotin. Dilute 100 times in 1 × Wash Buffer before use.

Streptavidin-HRP (100×)	PNH002	1 × 140 μL	HRP conjugated to Streptavidin. Dilute 100 times in 1 × Wash Buffer before use.
TMB Substrate	PND002	1 × 12 mL	Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	1 M hydrochloric acid. Avoid direct contact with eyes, skin, and clothing.
Sealing Film	PNK001	3 pieces	Cover the strips with it during incubation to prevent contamination and liquid evaporation.

Note: Room temperature refers to 25±3℃.

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as shown in Table 2.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-TrypsinMicrotiter Strips	Store in the bag with desiccant at 2-8℃ for up to 90 days.
Reconstituted Trypsin Calibration Standard	Store below -20℃ for up to 90 days. Freeze and thaw no more than 3 times.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel micropipettes: 1000 μL, 100 μL and 10 μL
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

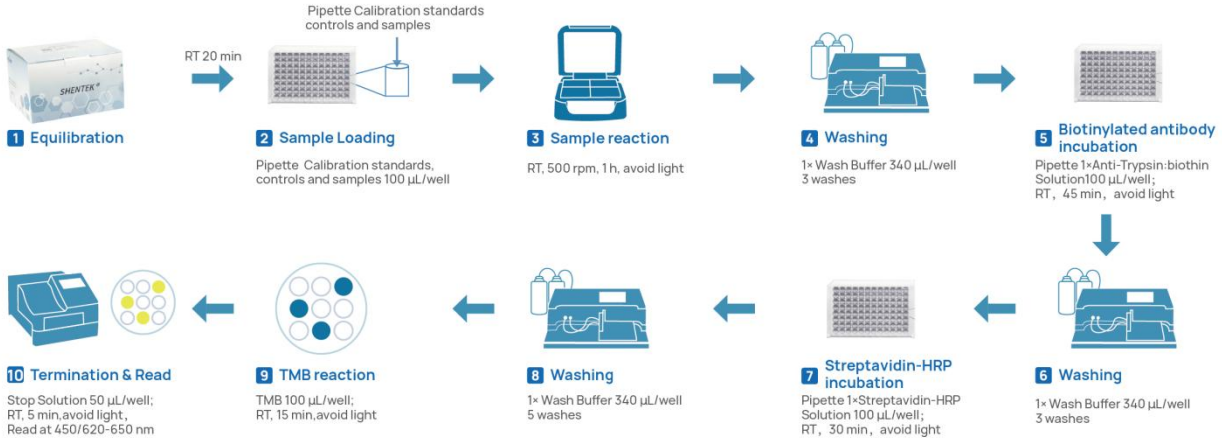


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Return to 2-8℃ after use.
- Take the appropriate amount of strips to a strip holder according to your experimental design and store the remaining strips in the vacuumed bag with desiccant at 2-8℃.

(2) Preparation of Reagents

- Trypsin Calibration Standard solution: Pipette 100 µL of Reconstitution Solution into the bottle containing Trypsin Calibration Standard. use the vortex oscillator and microcentrifuge for 3 times to mix well and let it stand for 5 minutes. Save the remaining solution under the recommended condition.

  Note: Do not use any other volumes of Reconstitution Solution to dissolve the Calibration Standard.

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to prepare 250 mL of 1×Wash Buffer. Prepare fresh and mix well before use.

  Note: If the Wash Buffer Concentrate (10×) is cloudy or contains precipitates,

heat at 37℃ until it clears.

(3) Preparation of Calibration Standard Solutions

- Prepare Trypsin Calibration Standard Solutions as indicated in Fig 3 and Table 3.

Figure 3. Graphic scheme of Trypsin Calibration Standard Solutions

Table 3. Preparation of Trypsin Calibration Standard Solutions

Tubes	Dilution procedure	Conc. (ng/mL)
ST1	Dilute the Trypsin Calibration Standard to ST1 with 1×Wash Buffer	2.56
ST2	500 µL ST1 + 500 µL 1×Wash Buffer	1.28
ST3	500 µL ST2 + 500 µL 1×Wash Buffer	0.64
ST4	500 µL ST3 + 500 µL 1×Wash Buffer	0.32
ST5	500 µL ST4 + 500µL 1×Wash Buffer	0.16
ST6	500 µL ST5 + 500 µL 1×Wash Buffer	0.08
ST7	500 µL ST6 + 500 µL 1×Wash Buffer	0.04
NCS	1×Wash Buffer	0

Note: We recommend to dilute the Trypsin Calibration Standard 10 times first, for the example above, add 10 µL of Trypsin Calibration Standard to 90 µL of 1×Wash Buffer, the ST1 is prepared with the 10 times diluted Trypsin Calibration Standard.

(4) Sample Preparation

- Test samples: Harvested bulk, in-process samples, drug substance and drug product. Samples should be clear and transparent, and insoluble substances need to be removed from samples by centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thraw cycles. For long-term storage, -70°C is recommended to avoid degradation.
- Dilute the samples with a suitable diluent to achieve a proper Trypsin concentration within the range of the calibration curve.

- For the first use, a method validation is recommend to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample dilution series.

Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Loading

- Pipette 100 μL of Calibration Standard Solutions, controls and samples into the corresponding wells as prepared earlier. Avoid foaming bubbles during pipetting. We recommend to prepare 2-3 replicates for each sample.
- Seal the plate and incubate on microplate thermoshaker at 500 rpm for 1 hour (room temperature), and protect from light.

Table 4. Example of 96-well plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1		S1	S1	S1					
B	ST2	ST2	ST2		S2	S2	S2					
C	ST3	ST3	ST3		S3	S3	S3					
D	ST4	ST4	ST4		S1+SRC	S1+SRC	S1+SRC					
E	ST5	ST5	ST5		S2+SRC	S2+SRC	S2+SRC					
F	ST6	ST6	ST6		S3+SRC	S3+SRC	S3+SRC					
G	ST7	ST7	ST7									
H	NCS	NCS	NCS									

- ✧ “ST1-ST7” indicate 7 concentration gradients, “NCS” as negative control, “S1-S3” as test samples, and “S1+SRC-S3+SRC” as spiked recovery controls for each sample.
- ✧ The number of replicates and the spiked samples can be determined by a method validation study.

(2) Biotinylated Antibody Reagent Preparation and Incubation

- 1×Anti-Trypsin:Biotin: Prepare 1×Anti-Trypsin:Biotin solution by diluting the Anti-Trypsin:Biotin (100×) with 1×Wash Buffer in a sterile centrifuge tube. Mix the solution gently and use immediately.
- Wash the plate with 340 μL of 1×Wash Buffer per well. Repeat washing for 3

times. Wipe off any liquid from the bottom outside of the plate. Do not allow the wells to be completely dried before adding the 1×Anti-Trypsin:Biotin solution.

- Add 100 μL of 1×Anti-Trypsin:Biotin solution into the wells, then incubate at room temperature for 45 minutes and protect from light.

(3) Streptavidin-HRP Preparation and Incubation

- 1×Streptavidin-HRP: Prepare the 1×Streptavidin-HRP solution by diluting the Streptavidin-HRP (100×) with 1×Wash Buffer in a sterile centrifuge tube. Mix the solution gently and use immediately.
- Wash the plate with 340 μL of 1×Wash Buffer per well. Repeat washing for 3 times. Wipe off any liquid from the bottom outside of the plate. Do not allow the wells to be completely dried before adding the 1×Streptavidin-HRP solution.
- Add 100 μL of 1×Streptavidin-HRP solution into the wells, incubate at room temperature for 30 minutes and protect from light.

(4) TMB Reaction

- Equilibrate the TMB substrate for 20 min at room temperature.
- Wash the plate with 340 μL of 1×Wash Buffer per well. Repeat washing for 5 times. Wipe off any liquid from the bottom outside of the plate. Do not allow the wells to be completely dried before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, incubate at room temperature for 15 minutes and protect from light.

  Note : Do not use sealing film during this step.

(5) Termination

- Add 50 μL of Stop Solution into each well.

  Note: The order of adding Stop Solution should be the same as the order of adding the TMB solution. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.
- Incubate at room temperature for another 5 minutes, and protect from light.

(6) Reading

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

- The OD value of each well should be calculated by the difference between OD_{450nm} and their respective long wavelength. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- Subtract the OD value of the NCS from each calibration point and samples, and record the mean of the replicate wells.
- Perform a 4-parameter logistic regression model using the Calibration standard concentration values and OD values to obtain the calibration curve equation. Substitute the average OD value of the samples into the equation to calculate the samples concentration, which should be multiplied by the dilution factor to obtain the actual samples concentration.
- The software for data analysis of the standard curve could be the one that comes with the microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- For those sample absorbance values exceeded the Calibration Standard ST1, a pilot study should be performed to determine an appropriate dilution before retesting. The Trypsin concentration in the samples are calculated from the test value multiplied by their corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level, and the recovery rate should meet the requirements of the corresponding regulations.

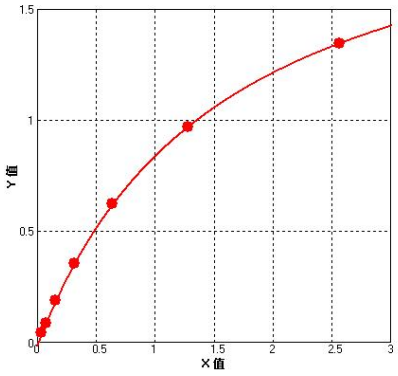
■ Limitations

- For research purposes only, but not intended for clinical use.
- The product is only applicable to the detection of Porcine Trypsin.
- Recommend samples pH between 6.0 and 8.0, beyond this range may cause abnormal results.

■ Assay Performance

- Linearity & Range: 0.040-2.56 ng/mL, $R^2 \geq 0.990$.
- LLOQ: 0.04 ng/mL.
- Typical calibration curve and data:

Calibration Standards (ng/mL)	Abs. At (450 nm-620 nm)	AVG
2.56	1.4213	1.4163
	1.4318	
	1.3959	
1.28	1.0131	1.0399
	1.1412	
	0.9654	
0.64	0.6985	0.6960
	0.7208	
	0.6686	
0.32	0.4234	0.4261
	0.4329	
	0.4220	
0.16	0.2584	0.2609
	0.2673	
	0.2571	
0.08	0.1512	0.1575
	0.1659	
	0.1553	
0.04	0.1124	0.1142
	0.1142	
	0.1161	
0.00	0.0672	0.0703
	0.0740	
	0.0697	



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A = 2.16282

B = -1.00528

C = 1.54458

D = -0.01333

R^2 =0.99996

- Specificity: No cross-reactivity with CHO, MDCK, Vero, HEK293, *E. coli* and *hansenula polymorpha*.

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe the pipette with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix different batches of kits.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-Trypsin:Biotin(100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples, for example, a minimum volume of 5 μL is recommended.
- ✧ Trypsin Calibration Standard Solutions, Biotinylated Antibody Solution and Streptavidin-HRP Solution are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ Centrifuge Anti-Trypsin:Biotin (100×) and Streptavidin-HRP (100×) before use to avoid any loss of the reagent.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assitance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Bubbles can influence optical density values and detection results.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of Trypsin evels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including distilled water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including micropipettes and centrifuge	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the Wash Buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Add the samples to the bottom of the well using micropipettes, and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- ICH. M10. Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation Guidance for Industry
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples

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Support & Contact

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