# Trypsin-analog ELISA Kit
# (One-step ELISA)
# User Guide

**PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT**

Product No.: 1402424
Version: A/1
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

## Product Name

Trypsin-analog ELISA Kit (One-step ELISA)

## Package

96 tests/Kit

## Intended Use

This kit is intended for determining the presence of Trypsin-analog in the biological product preparation process, and it is suitable to detect residue enzymes in this group such as TrypLE™ from Thermo, Recombinant Trypsin from Sartorius and HyrTryp™from Cytiva.

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

## Product Description

Trypsin-analog are a non-animal origin high-purity recombinant fungal enzyme that is obtained from fermentation production of *Fusarium oxysporum* DSM2672. Trypsin-analog cleaves peptides on the C-terminal side of lysine and arginine residues as standard trypsin solutions do, which can be used to dissociate a variety of adherent cells, including but not limited to HEK293, A549, Normal Human Epidermal Keratinocytes (NHEKs) and Embryonic Stem Cell (ESC).

This kit is based on the solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual Trypsin-analog in the samples. A sheep polyclonal antibody specific to Trypsin-analog was employed in the assay to capture any remaining Trypsin-analog in the samples. Both the Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled anti-Trypsin-analog antibody were simultaneously added to the microtiter plate, which coated with the affinity purified capture antibody and followed by incubation and washing. Then 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-analog concentration in the Calibration Standard and the samples. The concentration of Trypsin-analog in the samples can be calculated using the dose-response curve.

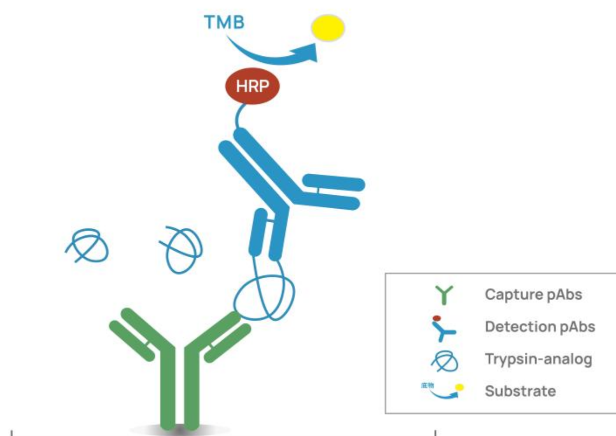



Figure 1. Schematic diagram

## ■ Kit Contents

Table 1. Kit Components

| Reagent | Part No. | Quantity | Note |
|---|---|---|---|
| Trypsin-analog Calibration Standard | PNB009 | 1×100 µL | The solution should be clear and transparent. Please refer to the label on the bottle for details. |
| Anti-Trypsin-analog Microtiter Strips | PNA009 | 8-well ×12 strips | Strips pre-coated with sheep anti-Trypsin-analog polyclonal antibody in a vacuumed bag with desiccant. Seal and store immediately after use. |
| Diluent | PNE005 | 2×25 mL | For dilution of Calibration Standard, Anti-Trypsin-analog:HRP (100×) and the samples. |
| Wash Buffer Concentrate (10×) | PNF001 | 1×25 mL | Easy to be crystallized at low temperatures, and can be dissolved in water bath at 37℃ before use. Dilute at 1:10 with freshly prepared ultra-pure water for plate washing. |
| Anti-Trypsin-analog:HRP (100×) | PNN003 | 1×140 µL | Sheep polyclonal antibody conjugated to HRP. Dilute 100 times before use. |

| TMB Substrate | PND002 | 1×12 mL | Equilibrate to room temperature for 20 minutes before use. Sealed after use and keep away from light. |
|---|---|---|---|
| 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℃ (including opened components). Please check the expiration date on the labels.

## Materials Required But Not Supplied in the Kit

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL, 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, 10 μL
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

## ■ Workflow

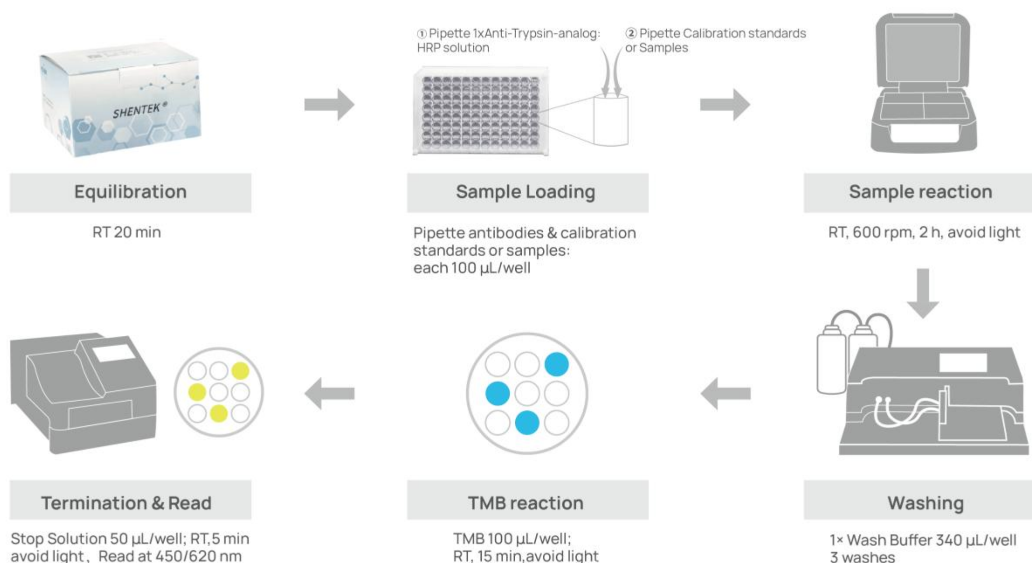



Figure 2. Procedure Flowchart

## 1. Preparation

### (1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes. Return to 2-8°C after use.
- Take the appropriate amount of strips to a strip holder according to the experimental design and store the remaining strips in the bag with desiccant at 2-8°C.

### (2) Preparation of Reagents

- 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×) or Diluent is cloudy or contains precipitates, heat at 37°C until it clears.
- 1×Anti-Trypsin-analog:HRP: Prepare the 1×Anti-Trypsin-analog:HRP by diluting the Anti-Trypsin-analog:HRP (100×) with Diluent in a sterile centrifuge tube. Mix the solution gently and use immediately.

## (3) Preparation of Calibration Standard Solutions

- Prepare Trypsin-analog Calibration Standard Solutions as indicated in Fig 3 and Table 2.

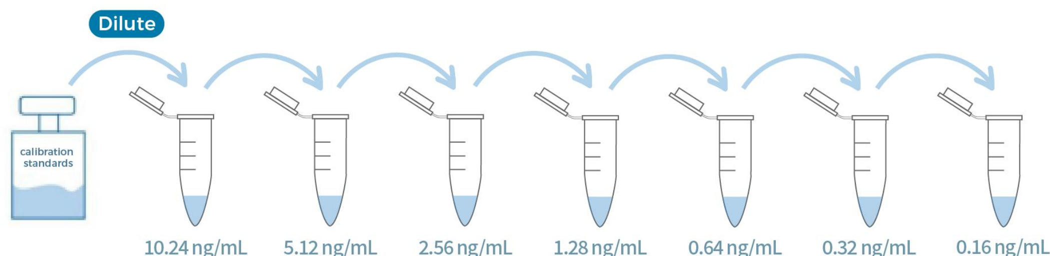



Figure 3. Graphic scheme of Trypsin-analog Calibration Standard Solutions

Table 2. Preparation of Trypsin-analog Calibration Standard Solutions

| Serial Dilution Tube | Dilution procedure | Conc. (ng/mL) |
|---|---|---|
| ST1 | Dilute the Trypsin-analog Calibration Standard to ST1 | 10.24 |
| ST2 | 500 µL ST1 + 500 µL Diluent | 5.12 |
| ST3 | 500 µL ST2 + 500 µL Diluent | 2.56 |
| ST4 | 500 µL ST3 + 500 µL Diluent | 1.28 |
| ST5 | 500 µL ST4 + 500µL Diluent | 0.64 |
| ST6 | 500 µL ST5 +500 µL Diluent | 0.32 |
| ST7 | 500 µL ST6 +500 µL Diluent | 0.16 |
| NCS | Diluent | 0 |

Note: It is recommended to dilute Trypsin-analog Calibration Standard 100 times, for the above example, add 10 µL of Trypsin-analog to 990 µL of Diluen. The ST1 is prepared with the 100 times diluted Trypsin-analog.

## (4) Sample Preparation

- Test samples: Cell harvest solution, 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 concentration of Trypsin-analog within the quantification range of the calibration curve.

- For the first use, 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 1×Anti-Trypsin-analog:HRP Solution into each designated well according to the experimental design.
- Pipette 100 μL of Calibration Standard, controls and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. It is recommended to prepare 2-3 replicates for each concentration.
- Seal the plate and incubate on microplate thermoshaker at 600 rpm for 2 hours (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 | NCS | NCS | NCS | | S1 | S1 | S1 | | | | | |
| B | ST7 | ST7 | ST7 | | S2 | S2 | S2 | | | | | |
| C | ST6 | ST6 | ST6 | | S3 | S3 | S3 | | | | | |
| D | ST5 | ST5 | ST5 | | S1+SRC | S1+SRC | S1+SRC | | | | | |
| E | ST4 | ST4 | ST4 | | S2+SRC | S2+SRC | S2+SRC | | | | | |
| F | ST3 | ST3 | ST3 | | S3+SRC | S3+SRC | S3+SRC | | | | | |
| G | ST2 | ST2 | ST2 | | | | | | | | | |
| H | ST1 | ST1 | ST1 | | | | | | | | | |

- ✧ "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 method validation.

### (2) Substrate Incubation

- Equilibrate the TMB substrate for 20 mins at room temperature.
- 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 dry before adding the substrate.

- Add 100 μL of TMB Substrate into the wells, and incubate at room temperature for 15 minutes, protect from light.

  Note: Do not use sealing film during this step.

### (3) 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, protect from light.

### (4) Reading

- Read absorbance at 450nm/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 sample into the equation to calculate the sample concentration, which should be multiplied by the dilution factor to obtain the actual sample 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, appropriate dilution should be performed before retesting. The Trypsin-analog

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.

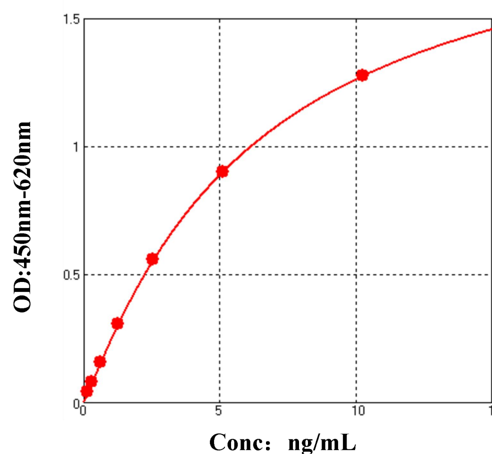
## Limitations

- For research purposes only, not intended for clinical use.
- Recommend sample pH between 6.5 and 8.5, beyond this range may cause abnormal results.

## Assay Performance

- Linearity & Range: 0.16-10.24 ng/mL, $R^2$>0.990.
- LLOQ: 0.16 ng/mL.
- Typical calibration curve and results for reference:

| Calibration Standards (ng/mL) | Abs. At (450 nm-620 nm) | AVG |
|---|---|---|
| 10.24 | 1.3199 | 1.317 |
| | 1.3051 | |
| | 1.3266 | |
| 5.12 | 0.949 | 0.944 |
| | 0.9476 | |
| | 0.9368 | |
| 2.56 | 0.6109 | 0.602 |
| | 0.5995 | |
| | 0.5955 | |
| 1.28 | 0.3575 | 0.352 |
| | 0.3582 | |
| | 0.3401 | |
| 0.64 | 0.2105 | 0.202 |
| | 0.1996 | |
| | 0.1969 | |
| 0.32 | 0.1270 | 0.124 |
| | 0.1221 | |
| | 0.1230 | |
| 0.16 | 0.0852 | 0.084 |
| | 0.0851 | |
| | 0.0812 | |
| 0 | 0.0425 | 0.041 |
| | 0.0409 | |
| | 0.0402 | |



- Specificity: No cross-reactivity with common engineering cell lines (e.g., MDCK, Vero, HEK293, *E.coli*, *P.pastoris* and Sf9 cells). And no cross-reactivity with protein analogs (e.g., recombinant human trypsin, recombinant bovine trypsin and recombinant porcine trypsin).

## ■ 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 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 the kit reagents from different lot numbers .
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37°C.
- ✧ 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-analog:HRP(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-analog Calibration Standard Solutions and anti-Trypsin-analog Antibody Solution are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ 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. Sometimes air, resulting in bubbles, can be drawn into the micropipette or dispensed into the wells. If this happens, bubbles can influence optical density values and results.
- ✧ Avoid the samples containing sodium azide ($NaN_3$), which will deactivate the HRP and lead to the underestimation of Trypsin-analog levels.

## ■ 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 sample 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
- ChP <9012> Guidance of Quantitative Method Validation for Biological Samples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## Support & Contact

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