TECHNICAL MANUAL

Trypsin Residual Contamination PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00006
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

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Trypsin Residual Contamination PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Assay Range:

0.039 to 2.5 ng/mL

Limit of quantification:

0.039 ng/mL

Limit of detection:

0.003 ng/mL

Detection Method:

Sandwich

Sample Type:

Biological samples

Precision:

CV% ≤ 10%

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit as indicated in section 4. Validity for 12 months . Date of expiration is on the ELISA Box label.

3. Product Description

The Trypsin Residual Contamination PharmaGenie ELISA Kit enables the quantitative measurement of residual trypsin in biological products. Trypsin is widely used in cell culture and protein processing to detach adherent cells, remove fusion tags, or facilitate protein digestion. However, incomplete removal of trypsin after processing can degrade therapeutic proteins, alter product stability, and trigger unwanted immune responses in patients. For these reasons, regulatory guidelines require strict monitoring of residual trypsin levels to ensure product safety, efficacy, and compliance.

This high-sensitivity assay employs a double-antibody sandwich ELISA enhanced with a streptavidin-biotin system for precise and reproducible detection. In the assay, microplate wells are coated with anti-trypsin capture antibodies to immobilize the enzyme. Samples are added, incubated, and washed, followed by a biotinylated detection antibody to form an antibody–trypsin–antibody complex. After an additional wash step, a streptavidin–HRP conjugate is introduced to complete the detection system.

The TMB substrate is then added, producing a blue color under HRP catalysis that turns yellow upon addition of the stop solution. The color intensity is directly proportional to trypsin concentration, with absorbance measured at 450 nm. Concentrations are determined from a standard curve, providing a sensitive, quantitative method for residual trypsin monitoring in compliance with quality control requirements.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	Coated microtiter plate	8 wells x 12 strips	Ready-to-use	Store 2 ~ 8°C
2	Biotinylated detection antibody (100x)	120 µL x 1 tube	Make a 100-fold dilution with diluent	
3	Streptavidin-HRP (100x)	120 µL x 1 tube	Make a 100-fold dilution with diluent	
4	Diluent	45 mL x 1 bottle	Ready-to-use	
5	Chromogenic solution	12 mL x 1 bottle	Ready-to-use	
6	Stop solution	6 mL x 1 bottle	Ready-to-use	
7	20x wash solution	35 mL x 1 bottle	Dilute with purified water at the volume ratio of 1:19 to obtain working solution for washing	
8	Standard (100 ng/mL)	0.5 mL x 1 tube	Dilute to desired concentration with diluent	
9	Sealing film	3 pieces	Ready-to-use	
10	Technical Manual	1 copy	-	

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

Note: *For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

7. Reagent Preparation

1. **Wash Buffer:** Take 1 portion of Buffer (20x), and add 19 times the volume of deionized water to prepare the Buffer at working concentration (1x). If there are crystals in the Wash Buffer (20x), shake gently at room temperature or in a 37°C water bath, and dilute after the crystals are completely dissolved. Unused Wash Buffer (20x) should be stored at 2 ~ 8°C.
2. **Preparation of standard:** : Dilute the standard to 50 ng/mL with 1× Buffer, and prepare the standard by 100-fold dilution, as shown in the figure above. Transfer 25 µL of the 100 ng/mL standard solution into 500 µL of sample dilution buffer to prepare the first standard at 2.5 ng/mL. Continue performing 1:100 serial dilutions by transferring 500 µL from the previous tube into 500 µL of fresh sample dilution buffer for each step. Prepare the remaining standards sequentially to achieve the following concentrations: 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, 0.156 ng/mL, 0.078 ng/mL and 0.039ng/mL. Mix each dilution thoroughly before proceeding to the next step. The prepared standards are used to generate the ELISA standard curve for quantitative analysis of test samples.
3. **Preparation of 100x biotinylated detection antibody and 100x streptavidin-HRP**: Dilute 100-fold with diluent 10 -15 min before use and equilibrate to room temperature before use.

8. Assay Procedure

1. **Equilibrate Reagents:** Bring each component in the kit to room temperature for 30 minutes. Take out required strip plates from aluminum foil bags already equilibrated to room temperature, and label the strip plate sequence with a marker. Seal remaining strip plates with a plate sealer, put them back to the aluminum foil bag, then seal the bag, and store at 2 ~ 8°C.
2. **Setup Plate:** Set the standard wells, blank wells and test sample wells, respectively. Recommended to assay samples in duplicate.
3. **Add Standard, Sample and Blanks:** Add standards at different concentrations (in sequence, blanks and samples to respective wells (100 µL/well). ***Note:*** *When it is not possible to determine the trypsin content in the sample to be tested, dilution factors shall be made with the diluent for the detection to avoid excessive content and inability to read valid values.*
4. **First Incubation:** Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 60 min
5. **Washing:** Discard the liquid, pat dry on absorbent tissue, fill each well with wash solution (300 µL), stand for 30 s, shake off the wash solution, pat dry on absorbent tissue, and repeat washing the plate 5 times in this way.
6. **Incubation of Streptavidin-HRP**: Add 100 µL of streptavidin-HRP at a working concentration to each standard well and sample well. Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 30 min
7. **Washing**: Same as Step 5.
8. **Colour development:** Add 100 µL of single-component substrate chromogenic solution to each well, seal the reaction wells with a sealing film, and allow them to stand at 37°C for 10 min in the dark.
9. **Reaction termination**: Add 50 µL of stop solution to each well and immediately perform the detection.
10. **Signal Reading**: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 20 minutes after reaction termination.

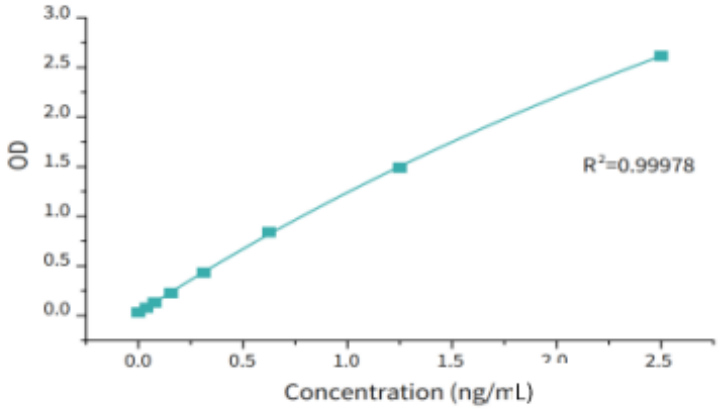
9. Data Analysis

The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.

OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail).

Std concentration (ng/mL)	OD Value (1)	OD Value (2)	Mean value
2.5	2.6273	2.6046	2.61595
1.25	1.5106	1.4703	1.49045
0.625	0.8347	0.8452	0.83995
0.3125	0.4358	0.4291	0.43245
0.156	0.2306	0.2232	0.2269
0.078	0.1307	0.1334	0.13205
0.039	0.0809	0.0765	0.0787
0	0.0325	0.0336	0.03305

The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.