Version 03-06/24

User's Manual

TrypLE TM ELISA Kit

REF **DEIA-JY2390**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The TrypLE^{TM} Quantitative ELISA Kit (enzyme-linked immunosorbent assay) is a high-sensitivity universal recombinant trypsin-like quantitation kit, capable of accurately identifying various recombinant non-animal sourced trypsin and its analogs. The kit can sensitively, specifically, and accurately detect residues of recombinant trypsin (TrypLE) in intermediates, semi-finished products, and finished products of various biological products. The detection range of this assay kit is 0.1-16 ng/mL, with a optimized quantitative limit of 0.1 ng/mL.

Principles of Testing

The ELISA kit uses a double-antibody sandwich enzyme-linked immunosorbent assay technique to quantify the trace residues of TrypLE in samples. Capture antibodies are coated onto a 96-well enzyme plate to create a solid-phase antibody. Subsequently, standard samples and test samples are added, followed by the addition of detection antibodies labeled with horseradish peroxidase (HRP), forming a solid-phase antibody-TrypLE-detection antibody sandwich complex. After the reaction, the plate is washed, and a substrate is added to initiate a color reaction. The substrate turns blue in the presence of HRP catalysis and ultimately turns yellow upon the action of a stop solution. Absorbance values (OD values) are measured at 450 nm and 630 nm wavelengths, with 630 nm serving as the correction wavelength. The OD value is positively correlated with the TrypLE content in the sample.

Reagents And Materials Provided

1. Microplate: 96 well polystyrene microplate (12 strips of 8 wells).
2. Standard: 1×300 µL, 1 µg/mL.
3. Detection Antibody: 1×15 mL, antibody conjugated to horseradish peroxidase (HRP) with preservatives.
4. Sample Dilution 20×: 30 mL of a 20-fold concentrated solution.
5. Buffer Concentrate: 30 mL solution.
6. Wash Buffer 20×: 30 mL of a 20-fold concentrated solution.
7. Chromogen Solution A: 8 mL, avoid direct light.
8. Chromogen Solution B: 8 mL, avoid direct light.
9. Stop Solution: 15 mL.
10. 3 Cover foil.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Incubator 37°C
3. Shaker
4. Precision pipettes to deliver 0.5 µl to 1 mL volumes

5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare the positive control or sample dilutions

Storage

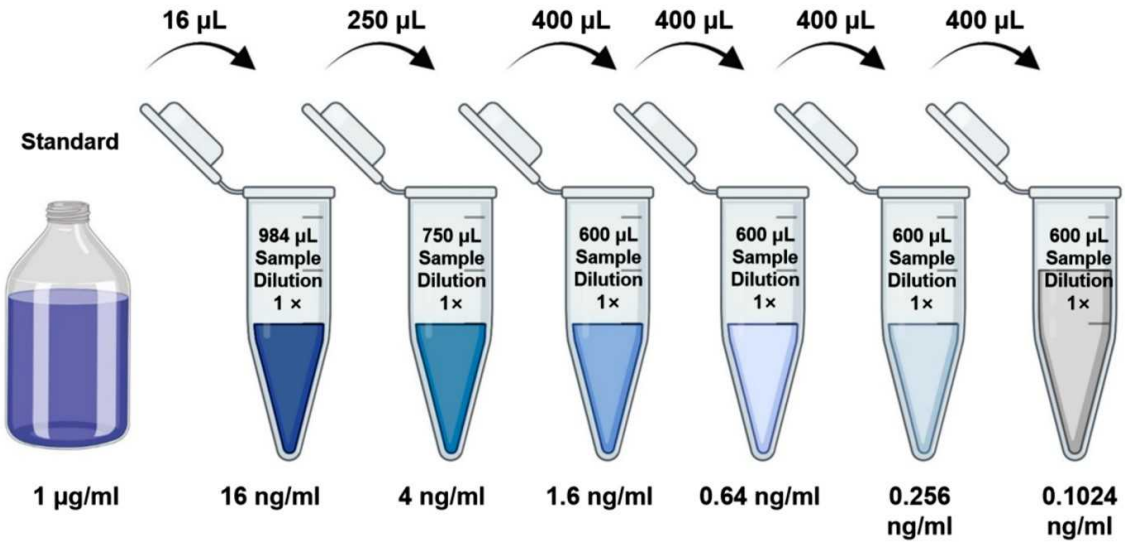
1. Store all reagents at 2-8°C, and not be frozen or thawed. The product is valid for 12 months.
2. All reagents must be brought to room temperature (20-25°C) prior to use.

Specimen Collection And Preparation

Equilibrate the samples to room temperature and mix before loading. If you need to dilute samples or the provided high-concentration standard solution, Buffer (1×) can be used for dilution. For cell samples, it is recommended to centrifuge at 3000 rpm for 5 minutes at room temperature, and then use the supernatant for testing before detection.

Reagent Preparation

1. Wash Buffer (1×): Prepare by Wash Buffer (20×) with deionized water at a 20-fold ratio, for example: mix 10 mL of Buffer (20×) with 190 mL of deionized water. Note: If crystals form in Buffer (20×), gently shake at room temperature or in a 37°C water bath until completely dissolved before dilution.
2. Chromogenic Solution: Mix equal volumes of Chromogenic Solution A and Chromogenic Solution B, then store away from light. Note: Do not store for too long; typically, prepare within 10 minutes before use. Do not use if the chromogenic solution turns blue after mixing.
3. Standard Solution: Prepare a new standard solution for each experiment. Add 16 µL of 1 µg/mL standard solution to 984 µL of Buffer (1×) to obtain a 16 ng/mL standard solution. Then, add 250 µL of 16 ng/mL standard solution to 750 µL of Buffer (1×) to dilute it to 4 ng/mL. Subsequently, dilute it according to the 2-fold ratio as shown in the figure below:



Assay Procedure

1. Allow all components of the ELISA kit to equilibrate at room temperature for 30 minutes, and it is recommended to perform duplicate measurements in all sample wells.
2. Remove the required strips from the aluminum foil bag, and mark the strip with a marker. Note: The strips are prone to detachment during the washing process, so Careful marking is important.
3. Sample incubation: Add the diluted standard and test samples to the plate (recommended: standard well, blank well, sample well; add the standard in concentration gradients) at 100 µL per well. Seal the plate with a sealing film and incubate at 37°C with 500 rpm shaking for 1 hour. (Note: Control the operating time within 10 minutes to avoid drift over time. Incomplete sealing or lack of sealing during incubation can lead to evaporation of the reaction solution, resulting in experimental errors.)
3. Washing: After incubation, carefully remove the sealing film, discard the liquid from the wells, wash the plate three times with wash solution (1×) at 250 µL per well, and pat dry the residual liquid in the sample wells. (If washing is done manually, allow the wash solution (1×) to stand for 1 minute; if using an automated washer, gently shake for 5 seconds after adding the wash solution.)
5. Incubation with detection antibody: Add the detection antibody to each well at 100 µL per well, seal the plate with a sealing film, and incubate at 37°C with 500 rpm shaking for 1 hour. (Note: Before adding liquid after each washing, check whether the strips are secured to prevent liquid splashing caused by strip displacement after liquid addition.)
6. Washing: Same as step 4.
7. Chromogenic Reaction: Add the pre-configured color development solution to the enzyme plate at 100 µL per well, seal the plate with a sealing film, and incubate at 37°C in the dark for 15 minutes.
8. Termination: Add the stop solution at 100 µL per well. Read the absorbance after the color has developed uniformly. (Note: Reading should be completed within 20 minutes after adding the stop solution).
9. Reading: Place the plate into the plate reader, set the wavelength to dual wavelengths at 450/630 nm, and read the absorbance values. (Note: It is recommended to set the plate reader program to include 5-10 seconds of shaking.)

Calculation

1. The calculation of the absorbance correction value for each standard or sample is:

   OD_{450nm} - OD_{630nm} - $OD_{Blank\ Control}$
2. Plot a standard curve with the concentration of the standard as the X-axis and the absorbance correction value of the standard as the Y-axis. It is recommended to use the four-parameter logistic mathematical model fitting equation:

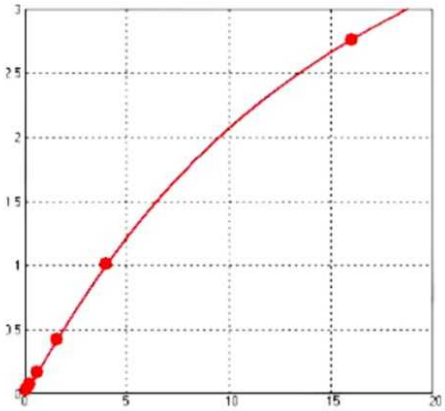
   Y = ((A-D)/(1+(X/C)^B)) + D

Substitute the absorbance correction value of the sample into the formula to calculate the content of TrypLE in the sample. (Pay attention to the dilution factor).

3. If the OD value of the sample exceeds the highest OD value on the standard curve, the sample needs to be diluted and retested.
4. Recommended dual-wavelength correction method. Use a wavelength of 630 nm for correction. The corrected OD value is OD_{450} - OD_{630}. This corrected value can be used directly for calculation or, based on

data quality, further blank correction can be performed. If a dual-wavelength microplate reader is not available, after reading the OD_{450}, the data quality should be evaluated before performing blank correction.

Typical Standard Curve



STD (ng/mL)	OD
16	2.7611
4	1.00655
1.6	0.42455
0.64	0.16745
0.256	0.0707
0.1024	0.02665

Performance Characteristics

Intra-assay Precision (Precision within an assay): CV% < 10%

Inter-assay Precision (Precision between assays): CV% < 15%

Spike Recovery: 70% - 130%

Detection Range

0.1 - 16 ng/mL

Detection Limit

< 0.1 ng/mL

Sensitivity

0.1 ng/mL