Version 09-04/24

User's Manual

Trypsin ELISA kit

REF **DEIA-NS2310-14**

Σ **96T**

RUO

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 test can be used to detect trypsin in biological samples

Principles of Testing

This kit adopts the principle of double-antibody sandwich method and is coupled with biotin-streptavidin system. The microwells of the microplate are coated with anti-trypsin antibodies, the sample is added, incubated and washed, and then biotinylated detection antibodies are added and incubated to form an antibody-antigen-antibody complex. After washing again, horseradish peroxidase (HRP)-labeled streptavidin was added. After thorough washing, the substrate TMB is added for color development. TMB is converted into blue under the catalysis of peroxidase, and is converted into the final yellow through acid termination. There is a positive correlation between the depth of color and the trypsin content in the sample. Use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm, and calculate the trypsin concentration in the sample based on the standard curve.

Reagents And Materials Provided

1. ELISA Microplate, 96 wells
2. Biotin-labeled Detection Antibody (Concentrated, 100×), 120µl
3. HRP-Streptavidin Conjugate(SABC, 100×), 120µl
4. Dilution Buffer, 45 ml
5. TMB Substrate, 12 ml
6. Stop Solution, 6 ml
7. Wash Buffer(20×), 35 ml
8. Standard (100ng/mL), 0.5mL
9. Plate Sealer

Materials Required But Not Supplied

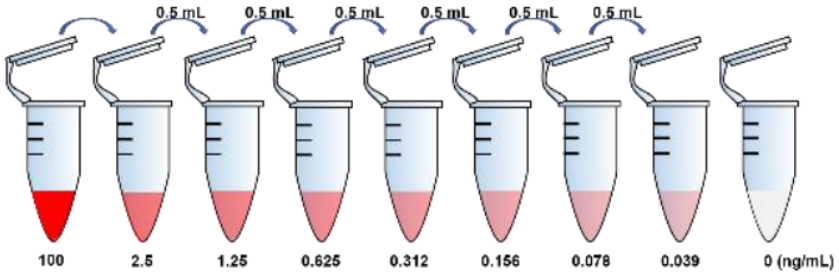
1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Microplate shaker

Storage

1. The kit is stored at 2~8°C, away from direct sunlight. It is valid for 6 months.
2. After the coating strips are opened and used, the remaining coating strips should be sealed, stored at 2~8°C, and used within the validity period.
3. After use, other components of the kit must be returned to 2~8°C in time and used within the validity period.

Reagent Preparation

1. Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C).
2. Dilute the 20× Wash Buffer into 1× Wash Buffer with pure water at a volume ratio of 1:19.
3. Dilute 100× Biotin-labeled Detection Antibody and 100× HRP-Streptavidin Conjugate 100-fold with Dilution Buffer 10-15 minutes before use. Equilibrate to room temperature before use.
4. Dilute the standard with Dilution Buffer to 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.3125ng/mL, 0.156ng/mL, 0.078ng/mL, 0.039ng/mL, 0ng/mL.



Assay Procedure

1. Remove the required strips from the aluminum foil bag after equilibration at room temperature. Cover the remaining strip with sealing film and seal it in a ziplock bag and return it to 2~8°C.
2. Set up standard wells and sample wells. Add 100 µL of standards of different concentrations to each standard well, and add 100 µL of the sample to be tested into the sample well. *When the trypsin content in the sample to be tested cannot be determined, the diluent should be used for multiple dilutions to prevent the content from being too high and unable to read a valid value.
3. Seal the reaction well with plate sealing film and shake the plate (500rpm) for 60 minutes at room temperature.
4. Discard the liquid and pat dry on absorbent paper. Fill each well with washing solution (300 µL), let it stand for 30 seconds, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times.
5. Add 100 µL of 1×Biotin-labeled Detection Antibody to each well of the standard well and sample well, seal the reaction well with a sealing film, and react with shaking plate (500 rpm) at room temperature for 60 minutes.
6. Discard the liquid and pat dry on absorbent paper. Fill each well with washing solution (300 µL), let it stand for 30 seconds, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times.
7. Add 100µL of 1×HRP-Streptavidin Conjugate to each well of the standard well and sample well, seal the reaction well with a sealing film, and react with shaking plate (500rpm) at room temperature for 30 minutes.
8. Discard the liquid, pat dry on absorbent paper, fill each well with washing solution (300 µL), let stand for 30 seconds, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times.
9. Add 100 µL of TMB Substrate to each well, seal the reaction well with a sealing film, and let stand at 37°C in the dark for 10 minutes.
10. Add 50µL of Stop Solution to each well and perform detection immediately. The wavelength of the microplate reader is 450nm (it is recommended to use dual wavelength 450nm/650nm).

Calculation

1. Take the average light absorption value of the standard, blank control, and sample, and subtract the average OD of the blank control to obtain the OD calibration value of the standard and sample. Create a standard curve with the concentration of the standard as the abscissa and the calibrated light absorption value of the standard as the ordinate. (Light absorption value in dual-wavelength detection mode is 450nm minus 650nm)
2. It is recommended to use professional curve making software, such as curve expert 1.3 or ELISA Calc (please use the 5-PL or 4-PL fitting curve calculation method to draw the standard curve), etc.

Precision

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

Inter-assay Precision (Precision between assays): CV%≤ 10

Detection Limit

1. Detection limit: 0.003ng/mL
2. Limit of quantitation: 0.039ng/mL

Specificity

This kit is used with CD's recombinant porcine trypsin. It can also be used to detect recombinant porcine trypsin or trypsin derived from pigs or cattle from other manufacturers. However, the corresponding product should be used to draw the standard curve.

Recovery

80%~120%

Precautions

1. The temperature and time after adding TMB Substrate are crucial to the experimental results and should be accurately grasped.
2. During the washing process, the reaction plate should be soaked in the washing solution for 30 seconds and then dried to fully wash the non-specifically adsorbed components.
3. All reagents should be shaken thoroughly before use. When adding samples, the added samples should be added to the bottom of the microplate to avoid adding them to the upper part of the well wall. When adding samples, be careful not to splash or generate bubbles.
4. If crystals are found in the concentrated washing solution, incubate it in a 37°C water bath. After the crystals are completely dissolved, mix and dilute it to the working concentration.
5. The introduction of sodium azide (NaN_3) should be avoided in the sample. Sodium azide will destroy the activity of horseradish peroxidase, causing the detection value to be low.

6. Before the experiment, please centrifuge the Biotin-labeled Detection Antibody and HRP-Streptavidin at 1000 rpm for 30 seconds to avoid residual reagents on the tube wall and tube cap.