Instructions for Use of Trypsin ELISA Detection Kit

This kit is intended for scientific use and not for diagnostic use

Cat. No. HG- TR001

Product introduction

This kit adopts the principle of double-antibody sandwich method and is coupled with a streptavidin-biotin system. Coat the microtiter plate wells with anti-trypsin antibodies, add samples, incubate and wash, and then incubate with biotinylated detection antibody to form antibody-antigen-antibody complex, and streptavidin (SA) horseradish peroxidase (HRP) conjugate is added after washing again. After washing again, add streptavidin (SA) horseradish peroxidase (HRP) conjugate. After thorough washing, the TMB substrate is added for chromogenic reaction, and TMB is converted to blue under the catalysis of peroxidase and finally to yellow by the termination effect of acid. The shade of color is positively correlated with the trypsin content in the sample. The absorbance (OD value) is measured with a microplate reader at a wavelength of 450 nm, and the trypsin concentration in the sample is calculated from the standard curve.

Detection range: 0.039 to 2.5 ng/mL

Limit of detection: 0.003 ng/mL

Limit of quantitation: 0.039 ng/mL

Precision: CV% ≤ 10%

Recovery rate: 80%-120%

Specification

96 T

Usage

Suitable for quantitative determination of trypsin content in samples.

Kit composition

Components	Specification	Preparation
Coated microtiter plate	8 × 12 plate strips	Ready-to-use
Biotinylated detection antibody (100×)	120 μL × 1 tube	Make a 100-fold dilution with diluent
streptavidin-HRP (100×)	120 μL × 1 tube	Make a 100-fold dilution with diluent
Diluent	45 mL × 1 bottle	Ready-to-use
Chromogenic solution	12 mL × 1 bottle	Ready-to-use
Stop solution	6 mL × 1 bottle	Ready-to-use
20×wash solution	35 mL × 1 bottle	Dilute with purified water at the volume ratio of 1:19 to obtain working solution for washing
Standard (100 ng/mL)	0.5 mL × 1 tube	Dilute to desired concentration with diluent
Sealing film	3 films	Ready-to-use
IFU	1 copy	Ready-to-use

Notes: Store the kit at 2-8℃.

Storage conditions and shelf life

The unopened kit is valid for 12 months at 2-8℃.

Materials to be self-prepared

(1) Microplate reader

(2) Microtiter plate constant temperature oscillator

(3) Pipettes and disposable tips (0.5-10 μL, 10-100 μL, 30-300 μL, 100-1000 μL)

(4) Deionized water

(5) Absorbent tissue

(6) EP tubes

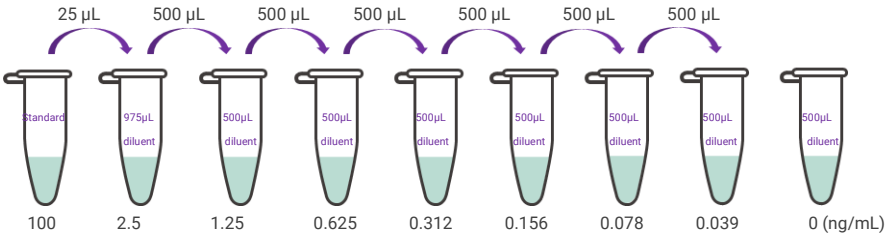
Preparation before experiment

(1) Equilibrate the kit to room temperature (18°C-25°C).

(2) 20× wash concentrate is diluted with purified water at a ratio of 1:19 to prepare working solution for washing.

(3) 100× biotinylated detection antibody and 100× streptavidin-HRP are diluted 100-fold with diluent 10 - 15 min before use and equilibrate to room temperature before use.

(4) Dilute the standard to 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.3125 ng/mL, 0.156 ng/mL, 0.078 ng/mL, 0.039 ng/mL, and 0 ng/mL in diluent.



Test steps

(1) Remove the required strips from the aluminum foil pouch after room temperature equilibration, seal the remaining strips with a sealing film and return to store at 2-8℃.

(2) Set the standard wells and sample wells, add 100 μL of standards at different concentrations to each standard well, and add 100 μL of samples to be tested to each sample well.

* 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.

(3) Seal the reaction wells with a sealing film and shake the plate (500 rpm) at room temperature for 60 min.

(4) 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.

(5) Add 100 µL of biotinylated detection antibody at 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 60 min.

(6) 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.

(7) 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.

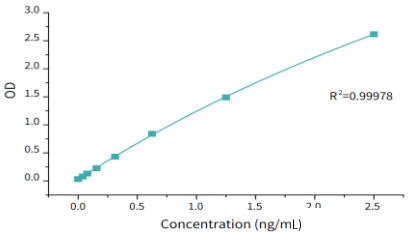
(8) Discard the liquid, pat dry on absorbent tissue, fill each well with wash solution (300 µL), stand for 30 s, shake off the wash solution, pat dry on absorbent tissue, and repeat washing the plate 5 times in this way.

(9) 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℃ for 10 min in the dark.

(10) Add 50 µL of stop solution to each well and immediately perform the detection. Set the microplate reader wavelength at 450 nm (it is recommended to use dual-wavelength 450 nm/650 nm).

Result processing

Standard curve:



Experimental Results:

Standard 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

Precautions

(1) The temperature and time for chromogenic reaction are essential for the experimental results and shall be accurately grasped.

(2) During the washing process, the wash solution shall be soaked in the reaction plate for 30 s and then shaken dry to fully wash the non-specific adsorbed components.

(3) All reagents shall be fully shaken well before use, and the sample added shall be added to the medium and bottom of the wells of the microtiter plate during sample addition to avoid adding to the upper part of the well wall, and attention shall be paid not to splash and bubbles during sample addition.

(4) If crystals are found in the concentrated wash solution, they can be incubated in a 37°C water bath, mixed and diluted to the working concentration after the crystals are completely dissolved.

(5) The introduction of sodium azide (NaN_3) shall be avoided in samples, which may destroy horseradish peroxidase activity and lower the detection value.

(6) Before the experiment, centrifuge the antibody tube and streptavidin-HRP tube at 1000 rpm for 30 s to avoid residual reagents on the tube wall and cap.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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