Trypsin Residue Detection Kit (ELISA)

Cat.No.: HYI134

Product Description

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Porcine trypsin antibody. Trypsin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Porcine trypsin antibody is added and binds to trypsin in the sample. After washing, HRP-Streptavidin is added and binds to the Biotinylated trypsin antibody. After incubation unbound HRP-Streptavidin is washed away. Then TMB substrate solution is added and catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of trypsin sample captured in plate. The absorbance is measured at 450 nm.

Product specifications

Recombinant Trypsin is frequently used in biopharmaceutical manufacturing-during cell preparation or for the modification and activation of products. Trypsin poses safety risks and must therefore be removed before final product release. This Sandwich Kit is for quantitative measurement of Residual Trypsin in cell culture supernatant and other procedures in biopharmaceutical manufacturing when Trypsin is used.

Components

Reagent	96 T
ELISA microplate	8×12 wells
Biotinylated detection antibody (100x)	120 µL
HRP-streptavidin (100x)	120 µL
Dilution buffer	45 mL
TMB substrate solution	12 mL
Stop solution	6 mL
concentrated wash buffer (20x)	35 mL
Standard (100ng/mL)	0.5mL

Sealing film	4 films

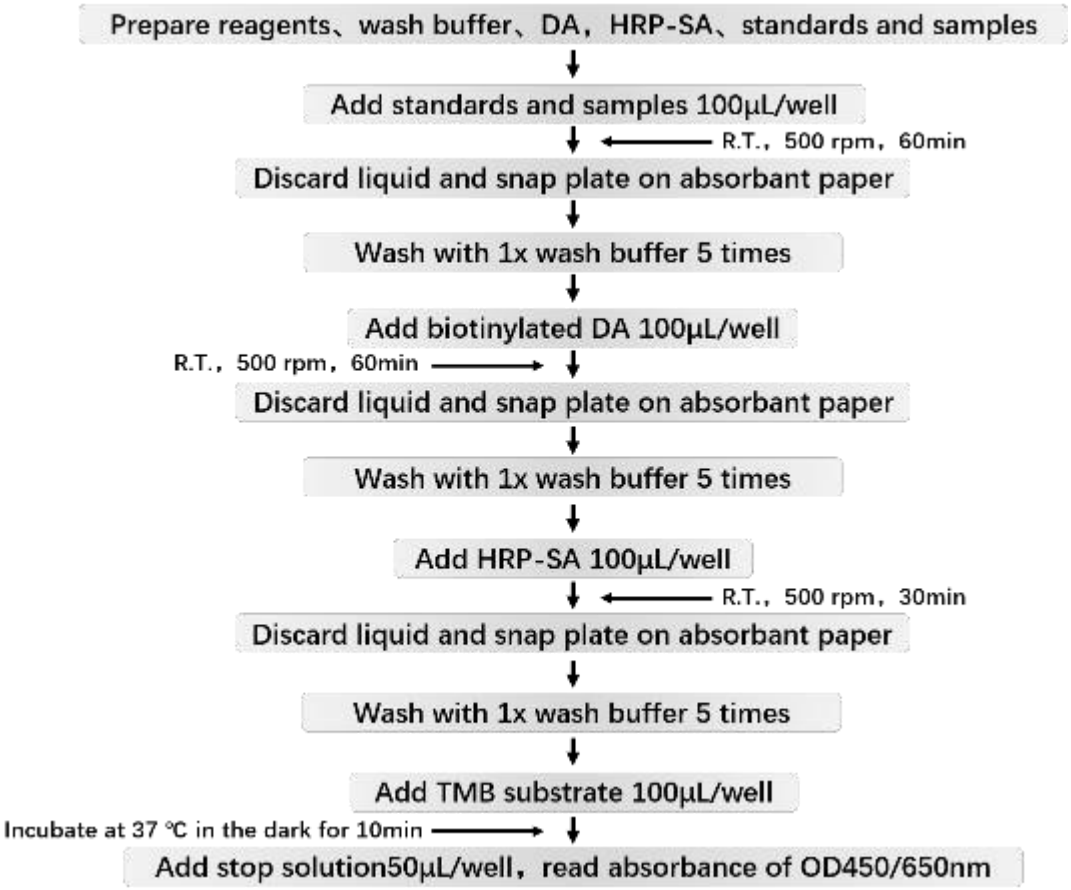
Transportation and storage

1. For unused kit: The whole kit could be stored at 2~8℃ in shelf life. Strong light should be avoided for storage stability.
2. For used kit: Once the microplate is opened, please cover unused wells with plate sealer and return to the foil pouch containing the desiccant pack, zip-seal the foil pouch and return to 2~8℃ as soon as possible after use. Other reagents should be returned to 2~8℃ as soon as possible after use.

Materials Required But Not Supplied

- Microplate reader with 450±10nm filter(better if can detect at 450 and 650 nm wavelength).
- Microplate shaker.
- 37℃ incubator.

Operation Flowchart



Instructions

1. Reagent preparation

1) Bring all kit components and samples to room temperature (18-25℃) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2) wash buffer: dilute 35mL of 20× concentrated wash buffer with 680mL of deionized or distilled water to prepare 700mL of 1× wash buffer.
3) standard: the concentration of the standard provided is 100ng/mL. Please firstly dilute the stock solution to 2.5ng/mL with dilution buffer and the diluted standard serves as the highest standard. Then prepare 7 tubes containing 0.5 mL dilution buffer and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.3125ng/mL, 0.156ng/mL, 0.078ng/mL, 0.039ng/mL and the last EP tubes with dilution buffer is the blank as 0ng/mL.
4) biotinylated detection antibody and HRP-streptavidin working solution: briefly spin or centrifuge the stock solution before use. Dilute them to the working concentration with dilution buffer.
5) TMB substate: aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. TMB substate is sensitive to light, don’t exposure TMB substrate to light for a long time.

2. Assay procedure

1) Determine the number of strips required for the assay. Insert the strips in the frames for use. Remaining plate strips not used in this assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.
2) Add 100μL each of dilutions of standard, blank and samples into the appropriate wells. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm. The samples should be diluted to appropriate concentration for accurate assay.
3) Wash step: Aspirate the solution and wash with 300μL wash buffer to each well and let it stand for 30s. Discard wash buffer completely by snapping the plate onto absorbent paper. Totally wash 5 times.
4) Add 100μL of biotinylated detection antibody working solution into each well. Cover with the plate sealer. Incubate for 1hr at room temperature with shaking at 500rpm.
5) Repeat wash step. 6. Add 100μL of HRP-streptavidin working solution into each well. Cover with the plate

6) sealer. Incubate for 30min at room temperature with shaking at 500rpm.
7) Repeat wash step again.
8) Add 100μL of TMB substrate solution into each well. Cover with the plate sealer. Incubate for 10 min at 37°C. Protect from light. The liquid will turn blue by the addition of substrate solution.
9) Add 50μL of stop solution into each well. The liquid will turn yellow by the addition of stop solution. Then run the microplate reader and conduct measurement at 450nm immediately.

3. Calculation of Results

1) Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve with absorbance on the vertical(Y) axis and dsRNA concentration on the horizontal(X） axis.
2) It is recommended to perform the calculation with computer-based curve-fitting software such as curve expert 1.3 or ELISA Calc in a 5 or 4 parameter non-linear fit model.

Performance characteristics

1. Sensitivity: lower limit of detection: 0.003ng/mL； lower limit of quantitation: 0.039 ng/mL
2. Precision: CV of Intra-Assay ≤10%， CV of Inter-Assay ≤15%
3. Recovery: 80%~120%
4. Specificity：this ELISA kit detects Trypsin, recombinant from porcine pancreas, expressed in *E.coli*, available from HYASEN (Cat No. HYI211/ HYI212). This kit can also detect recombinant Trypsin from other suppliers or Trypsin extracted from porcine or bovine, but the corresponding Trypsin should be used to make the standard curve.

Notes

1. TMB reaction temperature and time is critical, please control them according to the instruction strictly.
2. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
3. All the reagents should be mixed thoroughly prior to use and avoid bumbles during sample or reagents addition.
4. If crystals have formed in the concentrated wash buffer(20x), warm to 37°C and mix gently until the crystals are completely dissolved.
5. Avoid assay of samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of trypsin.

6. Before using the kit, centrifuge biotinylated trypsin antibody and HRP-streptavidin tubes at 1000rpm for 30s to bring down all components to the bottom of tubes.