ELISA kit for Trypsin

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Manual Version: V1.0

Product Name

EELISA kit for Trypsin

Size

HBP000211	96T/kit

Intended Use

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.

Assay Principle

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.

Kit Components

1.ELISA Microplate……..……..8×12

2.Biotinylated trypsin antibody

（200×）……..……75μL×1

3.HRP-streptavidin（100×）…....120μL×1

4.Dilution buffer.…….....45mL×1

5.TMB substrate solution.……12mL×1

6.stop solution…..……..…….6mL×1

7.concentrated wash buffer

（20×）…….…….……35mL×1

8.standard（100ng/mL）……0.5mL

9.plate sealer..……..……..…4 pieces

10.instruction manual.....1 copy

Storage of the kit

For unused kit: The whole kit could be stored at -25~-15℃ in shelf life，while up to one week at 2-8℃. For experiment convenience, reagents could also be stored separately, 96-well strip plate ,biotinylated trypsin antibody and HRP-SA should be stored at -25~-15℃ while the others could be at 2-8℃.

For used kit: when the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please cover unused wells with plate sealer and return to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

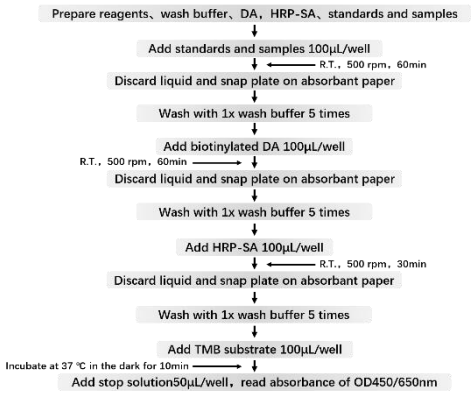
Materials required but not supplied

1.Microplate reader with 450±10nm filter(better if can detect at 450 and 650 nm wavelength).

2.37℃ incubator.

3.Shaker for microplates.

Operation Flowchart



Before you begin

1.Bring all kit components and samples to room temperature（18~25°C） 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 35 mL of 20× concentrated wash buffer with 700 mL of deionized or distilled water to prepare 900 mL 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 trypsin 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.

Assay Procedure

1.Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at -25~-15℃.

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.

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 trypsin 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 sealer. Incubate for 1hr 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℃. 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.

Calculation of Result

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 trypsin 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 parameter non-linear fit model.

Key Features and Details

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≤10%

3.recovery：80%~120%

4.specificity： this ELISA kit detects Trypsin, recombinant from porcine pancreas, expressed in Escherichia coli, available from Hzymes(Cat No. HBP000201-4/ HBP000206-9). Trypsin from other suppliers may show a different reactivity with regard to sensitivity and accuracy, therefore the compatibility of the kit calibration to the individual trypsin product must be verified.

Precautions

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℃ and mix gently until the crystals are completely dissolved.

5.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.

6.Avoid assay of samples containing Sodium Azide (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of trypsin.