Residual Porcine Trypsin ELISA
Cat No: KEP-0253

Background:
Porcine Trypsin Enzyme is frequently used in biopharmaceutical manufacturing - during cell preparation or for the modification and activation of products. Porcine Trypsin poses safety risks and must therefore be removed before final product release. Guideline No. EMA/CHMP/BWP/814397/2011 of EMA elaborates on the use of porcine trypsin used in the manufacture of human biological medicinal products

To ensure product safety, KinesisDX offers the Residual Porcine Trypsin ELISA kit to reliably detect complete and fragmented trypsin residues.

Principle:
This is enzyme-linked immunosorbent assay (ELISA) to detect the level of Trypsin in samples. Addition of controls or sample to Microtitre well which is pre-coated with Trypsin Anti -Porcine monoclonal antibody, if Trypsin present, it will bind to the Trypsin monoclonal anti-Porcine antibody coated on plate during incubation. After washing addition of HRP conjugate to form immune complex. Unbound HRP conjugate will get removed by washing step after incubation. Then addition of Substrate A and B, develops blue color during incubation period and reaction will get stop after addition of stop solution with development of yellow color. The concentration of the Trypsin of sample is directly proportional to the yellow color developed in well and will be positively correlated.

Intended Use:
The Kit is used to assay the level of Porcine Trypsin in cell culture supernatant. The Kit is For Laboratory / Research Use Only.

Materials provided in the kit:
1. Anti-Porcine Trypsin Coated Microtitre Plate (96 wells) – 1 no
2. Standard (Lyophilized) – 2 vial
3. Standard Dilution Buffer – 20 ml
4. Biotin-labeled Antibody (concentrated) - 120 ul
5. Antibody Dilution Buffer - 10 ml
6. HRP Conjugate – 120 ul
7. SABC Dilution Buffer - 10 ml
8. (25X) Wash Buffer – 30 ml
9. TMB Substrate – 10 ml
10. Stop Solution – 10 ml
11. Instruction Manual

Materials to be provided by the End-User:
1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Storage Information:
1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:
1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

2. Refer to the MSDS online for details.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN3, because NaN3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

   Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.

2. To make 1X Wash Solution, add 30 ml of 25X Wash Buffer in 720 ml of DI water.

3. Standard: Reconstitute the Standard with 1ml of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 100ng/mL Prepare 6 tubes containing 0.3mL Standard Diluent and use the diluted standard dilution series with 6 points; for example: 50 ng/mL 25 ng/mL 12.5 ng/ml, 6.25 ng/ml, 3.13 ng/ml, 1.56 ng/ml and the last tube with Standard Diluent is the blank at 0 ng/ml.

4. Biotin-labeled Antibody Working Solution: Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul Biotin-labeled antibody into 99ul Antibody Dilution Buffer).

5. HRP-Streptavidin Conjugate (SABC) Working Solution: Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer).

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Porcine Trypsin.
3. It is recommended that all Controls and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to poor sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1) Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.

2) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and standard diluent.

Standard	Standard No	Standard Dilution Particulars
50 ng/ml	Standard No.6	300 ul Original Standard + 300 ul Standard diluent
25 ng/ml	Standard No.5	300 ul Original Standard 5 + 300 ul Standard diluent
12.5 ng/ml	Standard No.4	300 ul Original Standard 4 + 300 ul Standard diluent
6.25 ng/ml	Standard No.3	300 ul Original Standard 3 + 300 ul Standard diluent
3.13 ng/ml	Standard No.2	300 ul Original Standard 2 + 300 ul Standard diluent
1.56 ng/ml	Standard No.1	300 ul Original Standard 1 + 300 ul Standard diluent

3) Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank.

4) Add 100ul each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 2 hours at 37°C.

5) Pipette out **100 ul** of **Biotin-labeled Antibody** into each sample well. Incubate for 1 hour at 37°C after covering it with the Plate sealer.

6) Pipette out **100 ul** of **Streptavidin-HRP Conjugate** into each sample and standards well

7) Cover the plate and incubate for 30 minutes at 37°C in the incubator.

8) Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

9) Then add **TMB Substrate 100 ul** to each well including Blank well. Gently mix and incubate for 10 min at 37°C in dark.

10) Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11) Read the absorbance at 450 nm within 30 minutes after adding the Stop Solution blanking on the zero standards.

Calculation of Results:

Calculate the mean optical density of each standard duplicate. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standards concentration on the X-axis. If ELISA software is being used, a 4-parameter curve is recommended. Calculate the mean optical density of each unknown duplicate. Read the values of the unknowns directly off the standard curve.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be **0.94 ng/ml**

Assay Linear Range: 1.56 - 50 ng/ml

LIMITED WARRANTY

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