Rat trypsinogen activation peptide (TAP) Elisa Kit

SKU: EK720903

Product Information

- Assay Type: Quantitative
- Species: Rat
- Standard: 2700 pg/ml
- Sensitivity: 12 pg/ml
- Detection Range: 33 pg/ml - 2000 pg/ml
- Sample Type: serum , plasma , urine , tissue homogenates , cell culture supernates
- Size: 96 Wells

Components

Assay plate (12 × 8 coated Microwells)	1
Standard (Lyophilized)	1×0.5ml
Standard Diluent	1×1.5ml
HRP-Conjugate Reagent	1×6ml
Sample Diluent	1×6ml
Chromogen Solution A	1×6ml
Chromogen Solution B	1×6ml
Stop Solution	1×6ml
Wash Solution	1×20ml×30 fold
User manual	1
Adhesive Strip	1

Test Principle

This ELISA kit uses Quantitative-ELISA as the method. The Micro-elisa strip plate provided in this kit has been pre-coated with an antibody specific to trypsinogen activation peptide. Standards or samples are added to the appropriate Micro-elisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for trypsinogen activation peptide is added to each Micro-elisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain trypsinogen activation peptide and HRP conjugated trypsinogen activation peptide antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of trypsinogen activation peptide. You can calculate the concentration of trypsinogen activation peptide in the samples by comparing the OD of the samples to the standard curve.

Sample Collection

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4oC before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8oC within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (Different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS
3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°Cfor15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg feces), sonicated(or mashed)and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

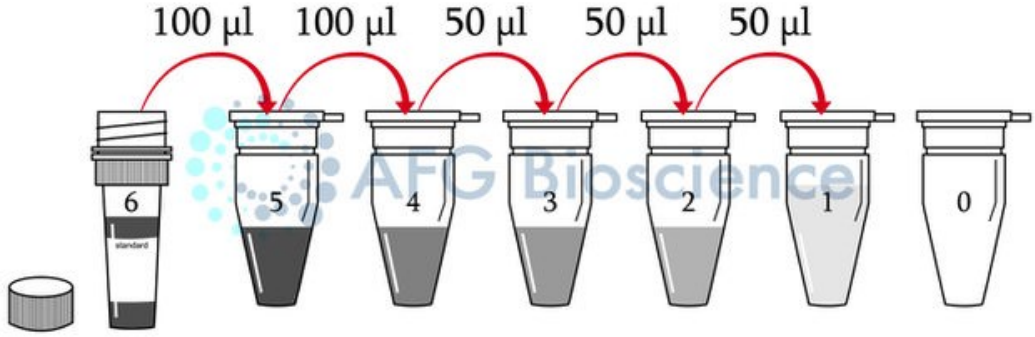
Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Reagent Preparation

1. Prepare wash buffer
    a. Combine 20 ml concentrated wash buffer (whole bottle) with 580 ml DI water
2. Standard Dilute the standard pipette 50 ul standard diluent in each tube. Pipette 100 ul standard in the fifth tube. And take out 100 ul from the fifth five tube into the fourth. Pipette 50 ul from the fourth tube to the third tube and produce dilution series as below. The undiluted Standard serves as the high standard. Sample Diluent serves as the zero standard blank well.



Tube	6	5	4	3	2	1	0
µmol/L	2700	1800	1200	600	300	150	0

Standard: 2250 pg/ml

Assay Procedure

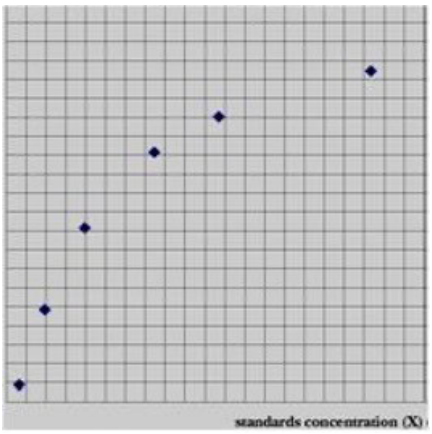
1. Prepare standards
    a. Pipette 100 ul standard diluent into new microcentrifuge tubes (labeled 5-0)
    b. Pipette 200 ul of standard into tube 5, vortex
    c. Transfer 200 ul of tube 5 into tube 4,
    d. Transfer 100 ul of tube 4 into tube 3 vortex repeat process to tube 1
    e. Tube 0 will only be sample diluent
2. Add 50 ul of standards in duplicate to provided 96-well plate
3. Using a multichannel pipette, add 40 ul of sample diluent to all wells that will contain samples (NOT WELLS CONTAINING STANDARD)
4. Add 10 ul samples to wells in duplicate
    a. Cover and incubate at 37°C for 40 minutes
5. Wash plate 5 times
    a. Adding 300 ul wash solution/wash
6. Add 50 ul of HRP to all wells EXCEPT blank (standard tube 0)
    a. Cover and incubate at 37°C for 40 minutes
7. Wash plate 5 times
    a. Adding 300 ul wash solution/wash
8. Add 50 ul of Chromogen A to all wells followed by 50 ul of Chromogen B
    a. Avoid light while preforming this step
    b. Cover and incubate at 37°C for 20 minutes
9. Add 50 ul of Stop solution to each well
10. Read immediately at 450 nm
11. When analyzing the plate, subtract the reading of the blank well from all other absorbances
    a. Additionally, a 5-fold dilution was done when samples were initially diluted so multiply concentration by 5 to get real concentration.

Calculation of Results:

Take the standard concentration as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, find out the corresponding concentration according to the sample OD value by the sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the

result is the sample actual concentration.

Graphical Representation as follows



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level trypsinogen activation peptide were tested 20 times on one plate.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level trypsinogen activation peptide were tested on 3 different plates, 8 replicates in each platez.

CV(%) = SD/mean X 100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Storage

Store kit at 2-8ºC immediately upon receipt. For long term the whole kit could be stored at -20 ºC in shelf life.

Download our ELISA technical hints, troubleshooting tips
www.afgsci.com/troubleshooting/

For technical support contact information, visit: https://www.afgsci.com/contact/