Rat trypsinogen activation peptide (TAP) ELISA Kit

Catalog No: EK720903

Reactivity	:	Rat
Method Type	:	Sandwich ELISA Detection
Quantity	:	96 tests
Sample Type	:	serum, plasma, Urine,tissue homogenates, cell culture supernates
Detection Range	:	33pg/ml - 2000 pg/ml
Sensivity	:	33pg/ml - 2000 pg/ml

Components:

Assay plate (12 × 8 coated Microwells)	1
Standard: 2250 pg/ml	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

Product Principle:

The kit is for the quantitative level of Rat TAP in the sample, adopt purified TAP antibody to coat microtiter plate, make solid-phase antibody, then add TAP to wells, Combine TAP antibody with labeled HRP to form antibody-antigen -enzyme-antibody complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of TAP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Specimen Requirements:

1.Serum-coagulation at room temperature for 10-20 mincentrifuge at the speed of 2000-3000 rpm for 20-min. Removesupernatant, if precipitation appeared, Centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoidrepeated freeze-thaw cycles.
2.Plasma-use suited EDTA or citrate plasma as an anticoagulant, centrifuge at the speed of 2000-3000 rpm for 20-min. Removesupernatant, if precipitation appeared, centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoidrepeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay .
3.Urine-collect sue a sterile container, centrifuge at the speed of 2000-3000 rpm for 20-min. Remove supernatant, if precipitationappeared, Centrifuge again. The Operation of Hydrothorax and cerebrospinal fluid reference to it. Assay immediately or aliquotand store samples at -20°C or -80°C.
4.Cell culture supernatant-detect secretory components, Remove particulates by centrifugation for 20-min at the speed of 2000-3000 rpm. Remove supernatant detect the composition of cells, dilute cell suspension with PBS (PH7.2-7.4), Cell concentrationreached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min atthe speed of 2000-3000 rpm. remove supernatant, If precipitation appeared, Centrifugal again. Assay immediately or aliquot andstore samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
5.Tissue samples- After cutting samples, check the weight, Pipette PBS(PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintainsamples at 2-8 after melting, Pipette PBS(PH7.4), homogenized by hand or Grinders, centrifugation 20-min at the speed of2000-3000 rpm. Remove supernatant. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.
Note:
1. Extract as soon as possible after Samples collection, and should be tested as soon as possible after the extraction. If not, samplesmust be stored at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.2. Can' t detect the sample which contain NaN3, because NaN3 inhibits HRP active.
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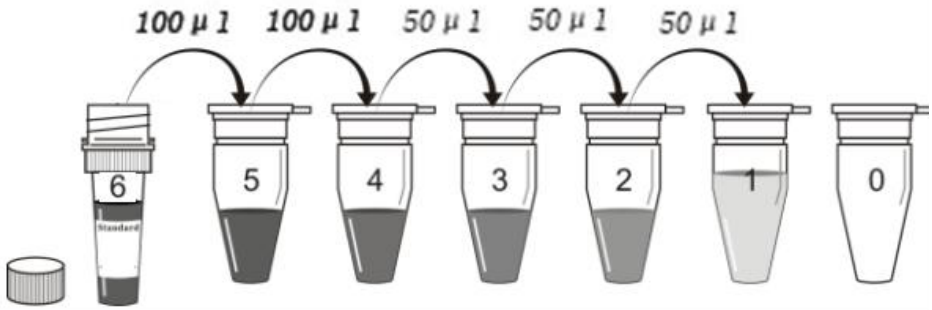
Reagent Preparation:

1:Wash Buffer (1x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (30 x) into deionized or distilled water to prepare 600 ml of Wash Buffer (1 x).
2:Standard Dilute the standardPipette 50l standard diluent in each tube. Pipette 100l standard (2250 pg/ml) in the fifth tube. And take out 100l from the fifth five tube into the fourth. Pipette 50l from the fourth tube to the third tube and produce dilution series as below. The undiluted Standard serves as the high standard (2250 pg/ml). Sample Diluent serves as the zero standardblank well(0 pg/ml).



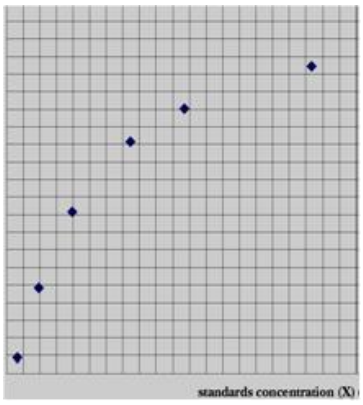
Tube	6	5	4	3	2	1	0
pg/ml	2250	1500	1000	500	250	125	0

Assay Procedure:

Step 1: Prepare all reagents, working standards, Blank and samples as directed in the previous sections.
Step 2: Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C
Step 3: Pipette standard 50l to testing standard wellPipette Sample diluent 40l to testing sample well, then add testing sample 10l (sample final dilution is 5-fold), Pipette sample to wells, don't touch the well wall as far as possible, and mix gently.
Step 4: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37.
Step 5: Configurate liquid: Dilute wash solution 30-fold with distilled water.
Step 6: Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times.
Step 7: Add enzyme: Pipette HRP-Conjugate reagent 50l to each well, except blank well.
Step 8: Incubate: Operation with 4
Step 9: Washing: Operation with 6.
Step 10: Color: Pipette Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, avoid the light preservation for 15 min at 37.
Step 11: Stop the reaction: Pipette Stop Solution 50l to each well, stop the reaction (the blue change to yellow). Step 12: Calculate: take blank well as zero. Read absorbance at 450nm after pipette Stop Solution within 15min.

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 following



Storage Conditions:

The unopened kit shall be stored at [2-8]
For opened kit can be stored at [2-8] for up to 1 month. If not be used recently, the standard should be kept in -20

Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat TAP were tested 20 times on one
plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Rat TAP were tested on 3 different
plates, 8 replicates in each plate.
CV(%) = SD/mean X 100
Intra-Assay: CV<8%
Inter-Assav: CV<10%

Attention:

1: The kit takes out from the refrigeration should be balanced 15-30 minutes in the room temperature, if the coated ELISA plates have not been used up after opening, the plate should be stored in sealed bag.
2: Washing buffer will Crystallization separation, it can be heated in water to dissolve.
3: Pipette sample with pipettors each step, and proofread its accuracy frequently to avoid the experimental error. Pipette sample within 5 min, if the number of sample is big, recommend using multichannel pipettor.
4: If the testing material concentration is excessively high (The sample OD is higher than the first standard well)please dilute the sample (n-fold).
5: Adhesive Strip only limits the disposable use to avoid cross-contamination.
6: The substrate should evade the light to be preserved.
7: Please refer to the user instruction strictly, the test result determination must take the microtiter plate reader as a standard.
8: The preparation of samples and all the reagents should refer to infective material process.
9: Do not mix reagents with those from other lots.

Washing Method:

Manually washing method: shake away the remained liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.
Automatic washing method: if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance .

If you have technical questions about our products, please email us to technical.support@afgsci.com and have all your questions answered.