SinoGeneClon Biotech Co.,Ltd

INTRODUCTION

Rat anti-double stranded DNA(dsDNA) ELISA Kit

FOR RESEARCH USE ONLY. Not for clinical diagnosis use

Catalog No : SG-20621

For the qualitative determination of Rat dsDNA concentrations.

Reactivity: Rat

Method Type: Sandwich ELISA Detection

Quantity: 96 tests

Sample type: serum, plasma, Urine,tissue homogenates, cell culture supernates

Components:

Assay plate (12 × 8 coated Microwells)	1
Positive control	1×0.5ml
Negative control	1×0.5ml
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	2

Product Principle:

The kit assay dsDNA in the sample, use purified antibody to coat microtiter plate, make solid-phase antibody, then add dsDNA to wells, Combined With dsDNA antibody which with HRP labeled become 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 sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. dsDNA ,after washing and removing non-combinative antigen and other components ,then Combined Compared with the CUTOFF value, according to this to judge dsDNA exist in the sample or not .

Specimen requirements:

1.Serum-coagulation at room temperature for 10-20 min，centrifuge at the speed of 2000-3000 rpm for 20-min. Remove supernatant, if precipitation appeared, Centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated 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. Remove supernatant, if precipitation appeared, centrifuge again. 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 .

3.Urine-collect sue a sterile container, centrifuge at the speed of 2000-3000 rpm for 20-min. Remove supernatant, if precipitation appeared, Centrifuge again. The Operation of Hydrothorax and cerebrospinal fluid reference to it. Assay immediately or aliquot and 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 concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 rpm. remove supernatant, If precipitation appeared, Centrifugal again. Assay immediately or aliquot and store 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, maintain samples at 2-8℃ after melting, Pipette PBS(PH7.4), homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 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, samples must 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.

Reagent preparation

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).

Assay procedure:

Step 1: Number: determine the number of well to be used and store unused wells in 4 ℃. Set a blank well without any solution

Step 2: Prepare sample: pipette Positive control and Negative control 50µl to the well respectively. Controls need test in duplicate. Pipette sample Diluent 40µl and testing sample 10µl to testing sample well. Pipette sample to the bottom of well, don't touch the wall as far as possible, and mix gently.

Step 3: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37℃.

Step 4: Configurate liquid: Dilute wash solution 30-fold with distilled water.

Step 5: Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times.

Step 6: Add enzyme: Pipette HRP-Conjugate reagent 50µl to each well, except blank well.

Step 7: Incubate: Operation with 3

Step 8: Washing: Operation with 5.

Step 9: Color: Pipette Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, avoid the light preservation for 15 min at 37℃.

Step 10: Stop the reaction: Pipette Stop Solution 50µl to each well, stop the reaction (the blue change to yellow).

Step 11: Calculate: take blank well as zero. Read absorbance at 450nm after pipette Stop Solution within 15min.

Calculation of result:

Test validity: the average of Positive control well≥1.00; the average of Negative control well ≤0.10.

Calculate Critical (CUT OFF): Critical= the average of Negative control well + 0.15.

Negative judgement: sample OD< Calculate Critical (CUT OFF) is dsDNA Negative .

Positive judgement: sample OD≥ Calculate Critical (CUT OFF) is dsDNA Positive .

Expiration:

Twelve months [see label on the outer box for the specific date]

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 ℃

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 Problems
Technical Service Contact information
Email: tech@sinogeneclon.com
Web: www.sinogeneclon.com
In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).