Human(NGAL)

ELISA Kit Instruction

Catalogue No.

201-12-1720D

Preface

Please carefully read this instruction before using. This ELISA kit is based on the principle of double-antibody sandwich technique to detect Human (NGAL). Be used only for research purposes, not be used for medical diagnosis.

Full Name

Human neutrophil gelatinase-associated lipocalin(NGAL)ELISA Kit

Intended Use

This kit is used to assay the neutrophil gelatinase-associated lipocalin (NGAL)in the sample of human’s serum, feces, plasma, saliva, urine and other related tissue Liquid.

Test principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human neutrophil gelatinase-associated lipocalin(NGAL)in samples. Add neutrophil gelatinase-associated lipocalin (NGAL)to monoclonal antibody Enzyme well which is pre-coated with Human neutrophil gelatinase-associated lipocalin(NGAL) monoclonal antibody, incubation; then, add (NGAL) antibodies labeled with biotin, and combined

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance neutrophil gelatinase-associated lipocalin(NGAL) of sample were positively correlated.

Materials supplied in the Test Kit

1	Standard (12.8ng/ml)	0.5ml
2	Standard diluent	3ml
3	Microelisa Stripplate	12well×8strips
4	Str- HRP-Conjugate Reagent	6ml
5	30×wash solution	20ml
6	Biotin-NGAL Ab	1ml
7	Chromogen Solution A	6ml
8	Chromogen Solution B	6ml
9	Stop Solution	6ml
10	Instruction	1
11	Closure plate membrane	2
12	Sealed bags	1

Materials required but not supplied

1. 37 ℃ incubator
2. Standard Enzyme reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

Important Notes

1. Beening taken out from the 2-8℃ environment, the kit should be balanced 30 minutes in the ambient temperature then use. If the Coated plates of Enzyme haven't been used up after opened, the remaining plates should be stored in Sealed bag.
2. For each step, add Sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
3. he operation shall be carried out accordance to the instructions strictly. And test results must be based on the readings of the Enzyme reader.
4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
5. All samples, washing buffer and each kind of reject should according to infective material process.
6. The idle agents shall be put up or covered. Do not use reagent with different batches. And use them before expired date.
7. The substrate B is light-sensitive. Prolonged exposure to light is forbidden.

Washing method

Manually washing method: shake away the remain 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

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

performance.

Specimen requirements

1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active

2. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.

3. serum- coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

4. plasma-use suited EDTA or citrate plasma as an anticoagulant, mix 10-20 mins , centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

5. Urine/Saliva-collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.

6. cell culture supernatant-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, detect the composition of cells, Dilut 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 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

7. Tissue samples- After cutting samples, check the weight, add PBS (PH7.2-7.4) , Rapidly frozen with liquid nitrogen, maintain samples at

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

2-8℃ after melting, add PBS (PH7.4), Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

8. Feces collection: using the clean bamboos to collect the abnormality feces with mucus, pus and blood, etc. For these feces with normal appearance, should collect samples from the surface, deep and ending of the feces. To get parasitemia and egg counts, should collect the feces within 24 hours. To detect the dysentery entamoeba histolytica trophozoites, should soon collect the soft part and with pus and blood, then detect immediately. To detect eggs of Schistosoma japonicum, should collect the mucus, pus and blood part, at least 30g feces is required for miracidium incubation, and please detect immediately. To avoid the Visible Components of the feces samples are damaged by digestive enzymes and pH, all feces samples should be detected well in 1 hour.

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

Assay procedure

1. Standard dilution:

this test kit will supply one original Standard reagent, please dilute it by yourself according to the instruction.

6.4ng/ml	Standard No. 5	120μl Original Standard + 120μl Standard diluents
3.2ng/ml	Standard No. 4	120μl Standard No. 5 + 120μl Standard diluents
1.6ng/ml	Standard No. 3	120μl Standard No. 4 + 120μl Standard diluent
0.8ng/ml	Standard No. 2	120μl Standard No. 3 + 120μl Standard diluent
0.4ng/ml	Standard No. 1	120μl Standard No. 2 + 120μl Standard diluent

2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

blank well. Every sample shall be made according to your required quantity, and try to use the duplicated well as possible.

3.Inject samples:

① Blank well: don’ t add samples and NGAL-antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A andB, and stop solution are allowed; other operations are the same.② Standard wells: add standard 50μl, Streptavidin-HRP 50μl(since the standard already has combined biotin antibody, it is not necessary to add the antibody);

③ To be test wells: add sample 40μl, and then add both NGAL-antibody 10μl and Streptavidin-HRP 50μl. Then seal the_sealing memberance, and gently shaking, incubated 60 minutes at 37 ℃.

4.Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.

5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.

6. Add chromogen solution A 50μl, then chromogen solution B 50μl to each well. Gently mixed, incubate for 10 min at 37℃ away from light.

7. Stop: Add Stop Solution 50μl into each well to stop the reaction(the blue changes into yellow immediately).

8. Final measurement: Take blank well as zero , measure the optical densit (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.

9. According to standards’ concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample’ s concentration. It is acceptable to use kinds of software to make calculations.

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

Summary procedures

Preparing reagents, samples and standards

⇩

Add prepared samples and standards, antibodies labeled with enzyme, reacting 60 minutes at 37 ℃

⇩

Plate washed five times, adding Chromogen solution A, B, reacting 10 minutes at 37℃

⇩

Add stop solution

⇩

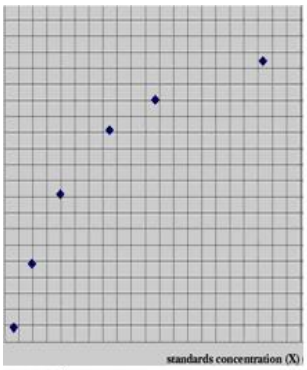
measure the OD value within 10min

⇩

Calculation

Calculate

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve (the result is the sample density)



sunred company
provides this chartis for reference only

or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density.

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103

Sensitivity、Assay range

Sensitivity:**0.02ng/ml**

Assay range:**0.3ng/ml→10ng/ml**

Specificity

This assay has high sensitivity and excellent specificity four detection of NGAL.No significant cross-reactivity or interference between NGAL and analogues was observed.

※ Limited by current skills and knowledge,it is impossible for us to complete the cross-reactivity detection between NGAL and all the analogues,therefore,cross reaction may still exist.

Package size

96T per box

Validity & Storage

6 months (2-8℃)

12 months (below -20℃)

www.srbooo.com TEL:86-21-66162175
Email:sunred@srbooo.com FAX:86-21-51564103