Rabbit TNF-α(Tumor Necrosis Factor Alpha) ELISA Kit

Cat# E4938

INFORMATION

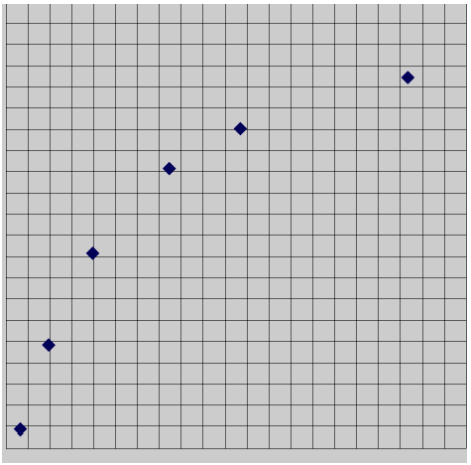
Catalog Number	E4938
Size	96T
Reactivity	Rabbit
Conjugate	HRP
Assay principle	This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibod
Sensitivity	9.38 pg/mL
Detection range	15.63—1000 pg/mL
Sample type	serum, plasma, cell lysates, tissue homogenates, cell culture supernatant or other biological fluids
Storage instruction	-20˚C and 4˚C for 6 months
Alias	DIF, TNF-alpha, TNFA, TNFSF2,TNF,TNFa
Purpose	This kit allows for the determination of TNF-α concentrations in Rabbit serum, plasma,tissue homogenates and other biological fluids.
Materials provided with the kit	

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Microelisa stripplate	1	1	2-8℃
Standard	0.3ml×6 bottle	0.3ml×6 bottle	2-8℃
HRP-Conjugate reagent	5ml×1 bottle	10ml×1 bottle	2-8℃
Sample diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
20×Wash solution	15ml×1 bottle	25ml×1 bottle	2-8℃

Note: Standard concentration was followed by:
320、160、80、40、20、0 pg/mL.

Specimen	1. **serum**- coagulation at room temperature 10-20 mins, centrifugation

requirements	20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. 2. **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. 3. **Urine**-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. 4. **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. 5. **Tissue samples**- After cutting samples, check the weight,add PBS PH7.2-7.4）,Rapidly frozen with liquid nitrogen, maintain samples at 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. 6. 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. 7. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active.
Assay procedure	1. **Add standard**: Set Standard wells, testing sample wells. Add standard 50μl to standard well. 2.**add sample**：Set blank wells separately (blank comparison wells don't add sample and HRP-Conjugate reagent, other each step operation is same). testing sample well. add Sample dilution 40μl to testing sample well, then add testing sample 10μl (sample final dilution is 5-fold), add sample to wells , don't touch the well wall as far as possible, and Gently mix. 3.**add enzyme**：Add HRP-Conjugate reagent 100μl to each well, except blank well. 4.**Incubate**: After closing plate with Closure plate membrane ,incubate for 60 min at 37℃. 5.**Configurate liquid**: 20-fold wash solution diluted 20-fold with distilled water and reserve. 6.**washing**：Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat. 7.**color**：Add Chromogen Solution A 50ul and Chromogen Solution B to each well, evade the light preservation for 15 min at 37℃ 8.**Stop the reaction**：Add Stop Solution 50μl to each well, Stop the reaction(the blue color change to yellow color).

	9.**assay**：take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.
Important notes	1. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag. 2. washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute . Washing does not affect the result. 3. add Sample with sampler Each step, And proofread its accuracy frequently, avoids the experimental error. add sample within 5 mins, if the number of sample is much ,recommend to use Volley . 4. if the testing material content is excessively higher (The sample OD is bigger than the first standard well),please dilute Sample (n-fold), Please diluente and multiplied by the dilution factor.（×n×5）. 5. Closure plate membrane only limits the disposable use, to avoid cross-contamination. 6. The substrate evade the light preservation. 7. Pleaseaccording to use instruction strictly, The test result determination must take the microtiter plate reader as a standard. 8. All samples, washing buffer and each kind of reject should according to infective material process. 9. Do not mix reagents with those from other lots.
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, multiplied by the dilution multiple, 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, multiplied by the dilution factor,the result is the sample actual density.
Assay range	10 pg/mL - 320 pg/mL