Rat TNF α ELISA KIT

Research Reagent

For in-vitro laboratory use only

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of Rat TNF (Tumor Necrosis Factor)-α with high sensitivity using Sandwich assay principle.

Advantage

(1) Rapid assay (total reaction time:4 hours.).
(2) A small sample volume (10µl in the standard procedure).
(3) An ecologically excellent preservative is used.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.

Components

	Reagents	Amounts
(A)	Antibody-coated plate	96 wells(8x12) / 1 plate
(B)	Standard rat TNF-α solution (30ng/ml)	100µl / 1 vial
(C)	Buffer solution	60ml/ 1 bottle
(D)	Biotin-conjugated anti-TNF-α	100µl/ 1 vial
(E)	HRP-conjugated streptavidin	100µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 vial
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 vial
(I)	Concentrated washing buffer (10x)	100ml/ 1 bottle

Assay sample

Rat serum or plasma : 10µl/well in the standard procedure.
(We recommend heparin (1.2~12U/ml) or EDTA (1mg/ml) for obtaining plasma.)
The volume of assay sample can be applied in the range of 10 ~ 50µl. In such case, the final volume of the liquid in the well should be adjusted to 50µl using assay buffer (C).
It would be convenient to dilute the assay samples first in test tubes, and pipette 50µl of the diluted sample to a well.

Assay range

16 ~ 3,000 pg/ml

Assay operation

1. Equipments necessary but not included in the kit.

(1) Micropipette (a micropipette able to deliver sample volume with high precision.), and a pipette for repetitive dispensing.
(2) Microplate washing apparatus (a microplate washer or a flashing bottle with a nozzle).
(3) A microplate reader (A densitometer for microplate).

2. Preparation of reagents

(1) Washing buffer:Dilute the Concentrated washing buffer (I) to 10X with purified water.
(2) Biotin-conjugated anti-TNF-α (D):Dilute to 100X with the Buffer solution(C).
(3) HRP- conjugated streptavidin solution (E):Dilute to 100X with the Buffer solution(C).
(4) Other reagents are used as they are.
(5) Use all the reagent solutions after bringing them up to room temperature (20-25C).

3. Preparation of standard solutions

(An example) Dilute the original standard solution (B) with the buffer solution to prepare 3,000pg/ml, then prepare lower standard solutions by a dilution program shown below.

Concentration. (pg/ml)	3,000	1,500	600	300	120	40	16	0

Std. Sol. (µl)	Orig.sol. 40	200*	200*	200*	200*	200*	200*	0
Buffer (µl)	360	200	300	200	300	400	300	200

*One rank higher standard solution

4. Assay procedure

***Remove the cover sheet of the microplate (A) after bringing up to room temperature.**

(1) Rinse the antibody-coated wells (A) 4 times by filling the washing buffer and discarding the buffer. Remove residual buffer in the wells by striking the plate upside-down onto several sheets of folded paper towel.
(2) Pipette 40µl of buffer solution into the wells for samples, then add 10µl of sample. Alternatively, if you use larger sample volumes (x µl), the volumes of buffer(C) should be (50-x) µl to adjust the final volume in wells to 50 µl.
It would be also convenient to dilute the assay samples first in test tubes, and pipette 50µl of the diluted samples to wells.
(3) Pipette 50µl of the standard solution to the assigned wells for preparing a standard curve.
(4) Shake the plate gently on a plate shaker.
(5) Incubate for 2 hours at room temperature (20-25C).
(6) Discard the reaction mixture, and then wash the plate 4 times as described in (1), and remove residual washing buffer remaining in the wells as in (1).
(7) Pipette 50µl of biotin-conjugated anti-TNF-α solution to all wells. Then shake gently on a plate shaker as in (4).
(8) Incubate the plate for 1 hour at room temperature.
(9) Discard the reaction mixture, and then wash the plate 4 times, and remove residual washing buffer as in (1)
(10) Pipette 50µl of HRP-conjugated streptavidin solution to all wells, and shake as (4).
(11) Incubate for 30 minute at room temperature.
(12) Discard the reaction mixture, and wash the plate 4 times, and remove residual washing buffer as in (1).
(13) Pipette 50µl of Chromogenic substrate reagent to wells, and shake as in (4).
(14) Let the plate stand for 30 minutes at room temperature.
(15) Add 50 µl of the Reaction stopper (H) to all wells and shake as in (4).
(16) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) within 30 minutes using a plate reader.

Summary of Assay Procedure

Antibody-coated plate (well)
↓
Washing 4 times
↓
Sample* or Standard 50µl
↓
Shake, then reaction for 2 hours at 20~25C
↓
Washing 4 times
↓
Biotin-conjugated anti-TNF-α 50µl
↓
Shake, then reaction for 1 hour at 20~25C
↓
Washing 4 times
↓
HRP-conjugated streptavidin 50µl
↓
Shake, then reaction for 30 min at 20~25C
↓
Washing 4 times
↓
Chromogenic substrate reagent 50µl
↓
Shake, then reaction for 30 min at 20~25C
↓
Reaction stopper 1M H_2SO_4 50µl
↓
Shake and measure absorbance at 450nm(sub. 620nm)

*Refer to the detailed procedure (2) for sample volume.

Calculation of TNF-α concentration

(1) Prepare a standard curve using normal or semi-logarithmic or bi-logarithmic section paper by plotting absorbance* (Y-axis) against standard concentration (pg/ml) on X-axis. For the manual reading from the standard curve, we recommend the use of bi-logarithmic section paper.
*Absorbance at 450nm minus absorbance at 620nm.
(2) Read TNF-α concentration of a sample from its absorbance*, and multiply the assay value by dilution rate (in the standard procedure, the dilution rate is 5) . Though the assay range is wide enough, in cases where the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.

We recommend the use of 3rd order regression curve or 4 parameter method in computer calculation. If you use logarithm transformation for TNF-α concentration and also absorbance, the fitness of the 3rd order regression will be improved.

Important notice in the treatments

1. Treatment of assay samples

(1) Use serum or plasma samples obtained by ordinary standard method.
(2) Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
(3) Measure the samples as soon as possible after sampling.
(4) It would be convenient to dilute the assay samples first in test tubes, and pipette 50μl of the diluted sample to a well.
(5) Do not use ether in anesthesia because it might influence assay reactions.

2. Storage of assay samples

If assay samples have to be stored for a long period, freeze samples and store below -35C. Avoid repeated freezing and thawing.

3. Influence of interfering substances

If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

Assay range and assay validation

1. An example of standard curve

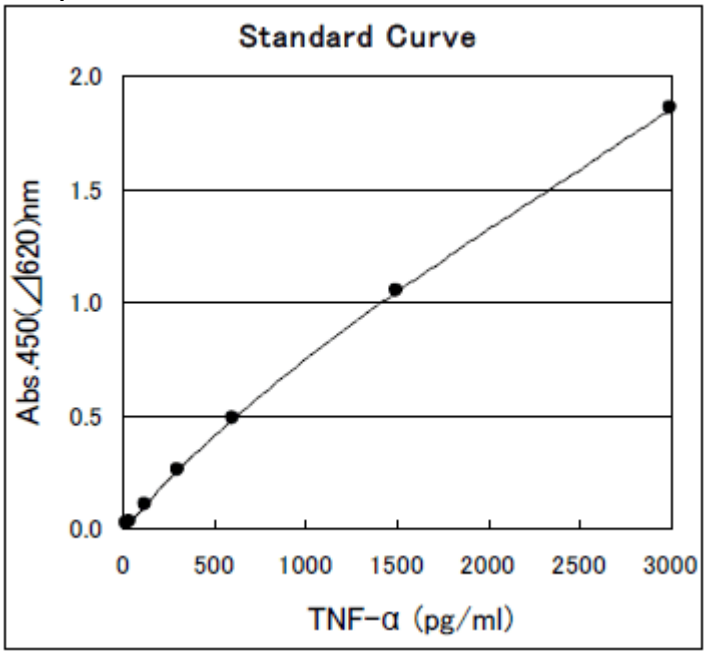

Plate-reader: Safire2(TECAN)

2. Specificity

Monoclonal antibodies which are highly specific to rat TNF-α are used.

3. Precision in 5 replicated assay, 2 samples

Within assay variation: Average CV is less than 5%.

4. Reproducibility in 4 replicated assay, 3 samples, 4 days)

Between assay variation: Average CV is less than 5%.

Notice

1. General caution on kits

(1) Place this kit keeping away from direct sunshine or heat.
(2) Do not freeze the kit.
(3) After opening the containers of reagents, use them as soon as possible. When storing, keep the tightly capped bottles under the temperature of 2~8C in a dark place.
(4) The reagents of the kit must not be used for other purpose than ELISA.
(5) Use fully dried lab-wares (pipette tips, glasswares) in assay.
(6) Temperature should be strictly kept in the range of 20~25C during assay process.

2. Precaution about kit and assay results

(1) Do not use the kit after its expiration date.
(2) The reagents were prepared specifically for each lot in order to give accurate results. So, do not combine the reagents in the kit of other lot number. Even if the lot number is the same, do not mix the reagents with those that have been preserved for some period.

Before starting assay, every reagent should be brought up to the room temperature (20~25C). Incomplete temperature condition may influence the assay results and cause incomplete performance of the kit.

(3) Judgment of the assay results should be carefully made from the overall situation including condition of the animals and results of other examinations.

3. Precaution in treatments

(1) In treating assay samples of animal origin, be careful for possible biohazards.
(2) After assay, samples and waists should be dipped in 1% formalin, 2% glutaraldehyde, or more than 0.1% sodium hypochlorite solution for more than 1 hour before discarding them. Alternatively, treat them in autoclave for sterilization.
(3) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Take special care when handling the stopping solution because it is 1M sulfuric acid.
(4) If such hazard happens, wash the place with enough volume of water, and if necessary, consult a doctor as soon as possible.

Storage condition

Store the kit at 2~8C. Do not freeze.

Term of validity

Six months from production. Expiration date is indicated on the container.

Unit of package

96-wells/1 plate

Product code

AKRTN-010

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