BioVendor
Research and Diagnostic Products

Mouse TNF-α ELISA

Cat. No.: RSHAKMTN011R

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of mouse 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 friendly 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 mouse TNF-α solution (30ng/ml)	100μl / 1 vial
(C)	Buffer solution	60ml/ 1 vial
(D)	Biotin-conjugated anti-TNF-α antibody	100μl/ 1 vial
(E)	Peroxidase-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

Mouse serum or plasma : 10μl/well in the standard procedure.
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 also convenient to dilute the assay samples first in test tubes, and pipette 50=l 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) 10-fold with purified water.

(2) Biotin-conjugated anti-TNF-α (D) : Dilute 100-fold with the buffer solution(C).
(3) Peroxidase-streptavidin solution (E): Dilute 100-fold with the buffer solution(C).
(4) Other reagents are used as they are.
(5) All the reagent solutions should be used after bringing them up to room temperature (20-25°C).

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.
(You can use other mode of dilution for a set of standard solutions.)

Concentration. (pg/ml)	3,000	1,500	600	300	120	40	16	0
Std. Sol.	Orig.sol.	200*	200*	200*	200*	200*	200*	0

(μl)	40							
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 getting back to room temperature.

(1) Rinse the antibody-coated wells (A) 4 times by filling the wells with 350μl of Wash 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 sample to a well.

(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 at 800-1,000 rpm for ~5-10s.

(5) Incubate for 2 hours at room temperature (20-25°C).

(6) Discard the reaction mixture, and then wash the plate 4 times as described in (1), and remove excess 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 as in (1), and remove excess washing buffer (1).

(10) Pipette 50μl of HRP-conjugated avidin 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, and remove excess washing buffer as in (1).

(13) Pipette 50μl of chromogenic substrate solution to wells, and shake as (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 within at 450 nm (sub-wave length, 620nm) within 30 minutes using a plate reader.

Summary of Assay Procedure

Antibody-coated 96 well plate

↓

Wash 4times

↓

Sample* or Standard 50μl*

↓

Shake then incubate for 2 hours at 20~25°C

↓

Wash 4 times

↓

Biotin-conjugated anti-TNF-α 50μl

↓

Shake then incubate for 1 hour at 20~25°C

↓

Wash 4 times

↓

Peroxidase-conjugated avidin 50μl

↓

Shake then incubate for 30 min at 20~25°C

↓

Wash 4 times

↓

Chromogenic substrate solution 50μl

↓

Shake then incubate for 30 min at 20~25°C

↓

Reaction stopper 1M H_2SO_4 50μl

↓

Shake then 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 (ng/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.

* 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.

(2) Read TNF-α concentration of a sample from its absorbance*, and multiply the assay value by dilution rate (in the standard procedure using 10μl of sample, the dilution rate is 5). Though the assay range is wide, 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.

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 also convenient to dilute the assay samples first in test tubes, and pipette 50μl of the diluted sample to a well.

2. Storage of assay samples.

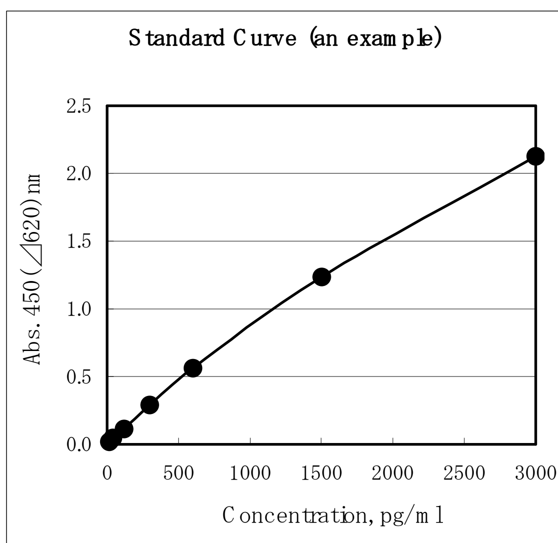
If assay samples have to be stored for a long period, freeze samples and store below –35°C. Avoid repeated freezing and thawing.

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. Model standard curves



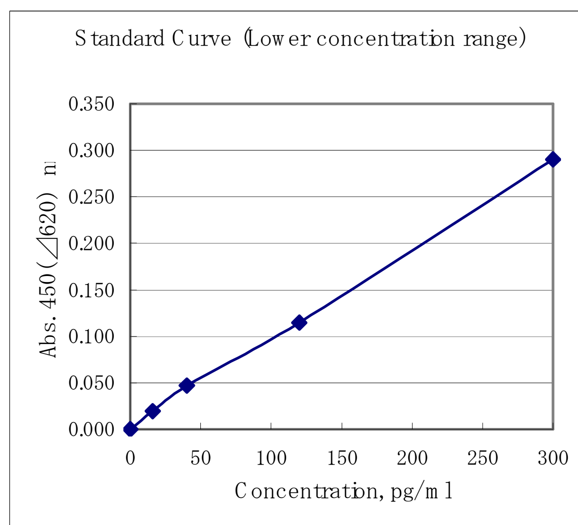


Plate-reader: SUNRISE RAINBOW (TECAN)

2. Specificity

Monoclonal antibodies which are highly specific to TNF-α are used. Crossreactivity to rat TNF-α is 56%.

3. Precision

Within assay variation

Wells	Samples	
	A	B
1	969	101
2	970	104
3	964	98.3
4	974	103
5	960	106
Mean	967	102
SD	5.41	2.76
CV(%)	**0.56**	**2.7**

Unit: pg/ml

4. Reproducibility

Between assay variation

Samples	Day 1	Day 2	Day 3	Day 4	Mean	SD	**CV (%)**
E	1495	1497	1511	1508	1503	8.03	**0.53**
F	300	299	301	304	301	2.15	**0.72**
G	42.3	42.1	42.2	43.2	42.4	0.54	**1.3**

Unit: pg/ml, n=4

5. Recovery test

Sample C

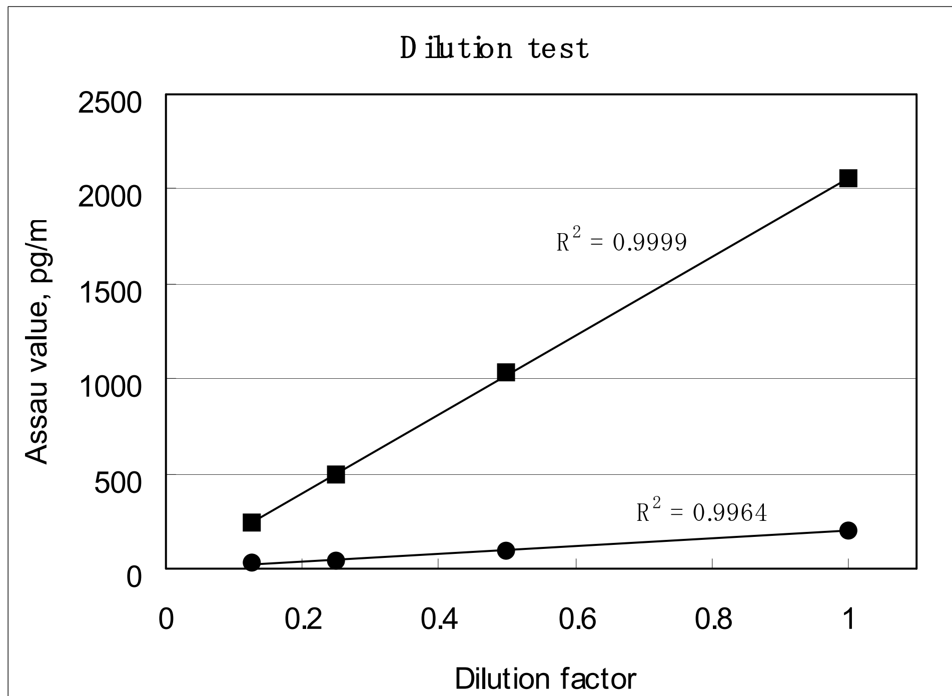
Added	Found	Recovered	Recovery (%)
0.00	34.9	-	-
15.7	49.5	14.63	**93.0**
23.6	58.6	23.8	**101**
47.2	85.8	50.9	**108**

Sample D

Added	Found	Recovered	Recovery (%)
0.00	325	-	-
283	617	292	**103**
550	879	555	**101**
625	915	591	**94.5**

Unit: pg/ml, n=2

6. Dilution test



Assay values of TNF-α in normal mice

Mouse: Balb/c、6 weeks old males from Charles River Laboratories Japan

Serum samples

ID	TNFα
1	143
2	72.3
3	59.4
4	93.2
5	71.3
6	62.9
7	84.9
8	44.8
9	52.4
10	20.2
11	121
12	30.6
13	53.9
Mean	75.1
SD	34.7

Unit: pg/ml, n=2

Influence of sample volume on assay value

Serum sample of Balb/c、6 weeks old male from Charles River Laboratories Japan

Sample volumes were adjusted according to the assay procedure(2).

	Volume	Wells			Mean	
Sample	μl	1	2	3	pg/ml	Sig.. Diff.
A	10	106	108	108	107	p>75%
	50	109	113	103	108	

No significant difference owing to sample volume.

Statements and precaution

(1) The reagents included in this assay kit should be used only for research works.

(2) The reagent solutions of the kit should be used immediately after reconstitution.

Otherwise, keep them in a dark place with the temperature 2-8°C, and use them within 3 days.

(3) 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.

(4) Pipetting and diluting of the reagent solutions should be made accurately because these steps influence the assay precision.

(5) Do not dry the assay plate to avoid denaturation of the coated antibody.

(6) Measurement of the reaction time should be started from the pipetting of reagent to the first well.

(7) Prepare the standard curve in each assay.

(8) Dilution of the assay sample must be carried out using ONLY the buffer solution included to the kit.

(9) Storage condition for the kit should be strictly followed.

(10) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Use special care when handling the stopping solution because it is 1M sulfuric acid.

(11) Avoid allowing the HRP-conjugated reagent solution, chromogenic substrate solution, to contact any metal.

(12) In treating assay samples of animal origin, be careful for possible biohazards.

(13) As the antibody-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

Storage condition

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

Term of validity

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

Unit of package

96-wells/1 plate

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