

**(For scientific research use only, not for clinical diagnosis!)**

**Canine Tumor Necrosis Factor  $\alpha$**   
**(TNFa) Quantitative Detection Kit**  
**(ELISA) Instructions for Use**

**Specification: 96T/48T Catalog Number:**

**SYP-C0012**

**Purpose: Used to detect the cause of canine tumor necrosis in serum, plasma, cell culture supernatant and other samples**

The concentration of sub-alpha (TNFa).



Please read the instructions carefully before use. If you have any questions, please contact us via:

**Official hotline: 400-999-8863**

**Technical phone number: 18358180525**

**Email: UpingBio@163.com**

**Company website: [www.upingbio.com](http://www.upingbio.com) For specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life. When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.**



## **[Kit performance]**

**Physical properties:** Each liquid component is clear and transparent, with no sediment or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

**Calibration curve linearity:** The correlation coefficient  $r$  value of the calibrator dose-response curve is greater than or equal to 0.9900.

**Precision:** intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%. **Sensitivity:** The lowest detectable dose is less than 7.812 pg/mL.

**Recovery rate:** The recovery rate is between 85%-115%.

**Sensitivity:** This kit recognizes natural canine tumor necrosis factor alpha (TNF $\alpha$ ) and has no crossover with structural analogs.

**Stability:** Stored at 2°C-8°C, validity period is 6 months.

**Detection range:** 31.25 pg/mL – 1000 pg/mL.



## 【Experimental principle】

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-canine tumor necrosis factor  $\alpha$  (TNFa) antibody (solid-phase antibody), add canine tumor necrosis factor  $\alpha$  (TNFa) calibrator and the sample to be tested, and then add biotin-labeled antibody. After incubation and sufficient washing, HRP-coupled avidin is added. After incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen-biotin-labeled antibody is formed on the solid surface of the microplate. -Sandwich complex of avidinase. Add TMB chromogenic solution to produce a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured on a microplate reader at a wavelength of 450nm. The absorbance (OD value) is related to the canine tumor necrosis factor in the sample to be tested. The concentration of  $\alpha$ (TNFa) is positively correlated. By fitting the calibrator curve, the concentration of canine tumor necrosis factor alpha (TNFa) in the sample can be calculated.





## [Kit components and storage]

| Components                    |                           | quantity  | Main                   |
|-------------------------------|---------------------------|-----------|------------------------|
| Calibrator                    | High Standard             | 2 vial    | Calibrator freeze-     |
| Calibration solution          | Reconstitution            | 2 vial    | PBS                    |
| Calibrators & Sample Diluents | Standard & Sample Diluent | 25mL      | PBSTN                  |
| coated microplate             | Microelisa Stripplate     | 96T/48T   | Pre-coated solid phase |
| biotin antibody               | Bio-Antibody              | 10mL      | biotin antibody        |
| HRP labeled avidin            | HRP-Conjugate             | 10mL      | HRP labeled avidin     |
| TMB chromogenic               | TMB                       | 10mL      | TMB                    |
| stop solution                 | Stop Solution             | 6mL       | acidic solution        |
| 20×concentrated               | 20X Wash Solution         | 25mL      | 0.05%Tween20           |
| manual                        | manual                    | 1 serving | --                     |
| Ziplock bag                   | Ziplock bag               | 1         | --                     |
| Self-adhesive                 | Self-adhesive             | 4 pieces  | --                     |

**Note: 1. Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.**

2. The test kit should be stored at 2-8°C. Expired test kits must not be used.
3. If the coated microplate is not used up in a single time, remember to seal it and store it at 2-8°C.
4. The reconstituted calibrator can only be used on the same day.

5. If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use.

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**Prepare your own test equipment required for the  
test (not provided, but can assist in purchasing) 1.**

Standard specification microplate reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

**[Kit limitations]**

1. For scientific research use only and not  
for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest calibrator concentration value,  
please dilute the sample appropriately and then re-measure. 6. Human anti-mouse

and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

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## 【Precautions】

1. This kit is for in vitro research only and not for clinical diagnosis.
2. Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.
3. Incubate strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.
4. Incorrect plate washing may lead to inaccurate results. Be sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
5. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
6. The substrate chromogenic solution should be colorless or very light in color.
7. Avoid cross-contamination of reagents and specimens to avoid erroneous results.
8. Avoid direct exposure to strong light during storage and incubation.

9. Open the sealed bag after balancing to room temperature to prevent water droplets from condensing on the cold slats.

10. Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11. The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of  $450\pm 10\text{nm}$ .

Optical density ranges from 0-3.5. It is recommended to preheat 15 minutes in advance before use.

12. Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.
13. The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.
14. Do not use expired reagents.

## **【Sample preparation and storage】**

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

**Cell culture supernatant: Centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.**

**Serum: Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.**

**Plasma: Anticoagulate with heparin, citrate or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood drawing. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.**

**Cell lysis medium: For adherent cells, remove the culture medium and replace with PBS, physiological saline or blood-free**



Wash with culture medium. Add an appropriate amount of lysis solution and pipet several times to ensure full contact between the lysis solution and cells.

Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, pipet with a pipette to disperse the cells, and flick with your fingers to fully lyse the cells.

After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

**Urine: Collect in sterile tubes and centrifuge at 2000×g for 20 minutes.**

**Carefully collect the supernatant. If a precipitate forms, centrifuge again.**

## **【Reagent preparation】**

1. Before use, all components must be rewarmed for at least 120 minutes to ensure full rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

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## **[Calibration dilution method]**

**Steps for redissolving the calibrator:** Redissolve the calibrator with the calibrator reconstituted solution. Add all the liquid in one bottle of the calibrator reconstituted solution to the bottle of calibrator freeze-dried powder. Vortex gently to ensure thorough mixing. , the mother solution concentration of the calibrator after redissolution is 2000 pg/mL , mix thoroughly before dilution.

**Dilution steps of the calibrator mother solution:** Let the calibrator working solution stand for 1-2 minutes before dilution. Use the calibrator & sample universal diluent to double dilute the calibrator mother solution. The doubling dilution method: take 7 EP tubes, and Add 500  $\mu$ L calibrator & sample diluent, pipet 500  $\mu$ L from the 2000 pg/mL calibrator mother solution into the first EP tube and mix to prepare a 1000 pg/mL calibrator working solution. Follow this step in sequence. Pipette and mix. As shown on the following page.



2000 pg/mL

**Recommended dilution concentration of calibrator: It is recommended to prepare the following concentrations: 1000, 500, 250, 125,**

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62.5, 31.25, 0 pg/mL, and used as the calibrator concentration value of the fitting standard curve.

**Tip: Add the calibrator & sample universal diluent directly to the last tube as the 0 value. There is no need to draw liquid from the penultimate tube. The diluted calibrator working solution needs to be prepared and used immediately.**

## **【Operating Procedure】**

**Recommended sample dilution scheme: It is recommended that teachers conduct preliminary experiments to explore the optimal dilution ratio of samples before conducting formal experiments.**

All reagents and components should be returned to room temperature first. It is recommended to perform duplicate wells for calibrators, quality control materials and samples.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.
2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up the calibrator hole, sample diluent hole, blank hole and sample hole. Add 50  $\mu$ L of calibrator of different concentrations to each of the calibrator holes. Add 50  $\mu$ L of sample diluent to the sample diluent hole. Do not add it to the blank hole. Add the sample hole to be tested. Sample 50 $\mu$ L. Except for the blank wells, add 100uL of biotin antibody to each well, cover the reaction plate with a sealing film, and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

4. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, and let it sit.

1 minute, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If using an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

5. Except for the blank wells, add 100uL of HRP-labeled avidin to each well, cover the reaction plate with a sealing film, and incubate in a 37°C water bath or incubator in the dark for 20 minutes.

6. Repeat step 4.

7. Add 100μL of TMB chromogenic solution to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450 nm wavelength microplate reader.





## 【Result calculation】

1. Use the concentration of the calibrator as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software] 2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.



(Schematic diagram, for reference only)



## [Operation Summary]



1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液, 37℃孵育60分钟。



2、弃掉板内液体, 洗板5次。



3、每孔加入100uL HRP酶结合物工作液 37℃孵育20分钟, 弃掉板内液体, 洗板5次。



4、每孔加入100uL TMB显色液, 37℃孵育 15分钟。



5、每孔加入 50uL 终止液。



6、立即在 450nm波长下读数, 处理数据。

**【problem analysis】**

| Problem Description                                | Possible Causes                          | Corresponding   |
|--|--|---|
| Negative and positive control results are unstable | Incorrect liquid                         | Check pipettes and tips   |
|  | Equilibration time is too                | Ensure sufficient   |
|  | Incomplete washing                       | Ensure the washing time and number of washes  |
| Very weak or colorless                             | Incubation time too short                | Ensure adequate   |
|  | Experimental                             | Use recommended   |
|  | Insufficient reagent                     | Check the liquid aspiration and addition  |
|  | Incorrect dilution                       |   |
|  | Enzyme label inactivation or substrate   | Mix enzyme conjugate and substrate and check  |
| Reading value is low                               | Microplate reader settings are incorrect | Check the wavelength and filter settings on the   |
|  |  | Turn on the microplate  |
| Large coefficient of                               | Adding fluid incorrectly                 | Check the filling   |
| High background value                              | The working                              | Use the recommended   |
|  | Incomplete washing of enzyme plate       | Ensure that each step of cleaning is complete; if using an automatic plate washer, please check |
|  | The lotion is                            | Prepare new lotion  |
| Low sensitivity                                    | Improper storage of ELISA kits           | Store relevant reagents according to instructions   |
|  | Not terminated before reading            | Stop solution should be added to each well  |

## UpingBio technology Co.,Ltd

If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the strips used and unused reagents, and then contact our company's technical support to solve the problem for you.

### **【statement】**

1. Due to existing conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.

3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the

use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.

6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products using different methods to detect the same target, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.







## 【Experimental experience】

## 【Experimental experience】