

ab193687 –TNF alpha Canine ELISA Kit

Instructions for Use

For the quantitative measurement of canine TNF alpha in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. **BACKGROUND**

Abcam's TNF alpha Canine ELISA Kit (ab193687) is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of canine TNF alpha in serum, plasma and cell culture supernatant.

This assay employs an antibody specific for canine TNF alpha coated on a 96-well plate. Standards and samples are pipetted into the wells and the immobilized antibody captures TNF alpha present in the samples. The wells are washed and biotinylated anti-canine TNF alpha antibody is added. After washing away any unbound biotinylated antibody, an HRP-conjugated streptavidin is pipetted to the wells. After incubation, the wells are again washed, followed by the addition of a TMB substrate solution to the wells. Color will develop in proportion to the amount of TNF alpha bound in each well. Addition of the Stop Solution will change the color from blue to yellow, and the intensity of the color is measured at 450 nm.

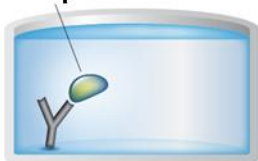
2. ASSAY SUMMARY

Primary capture antibody



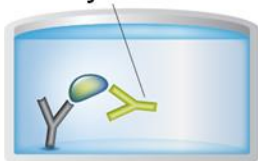
Prepare all reagents, samples and standards as instructed.

Sample



Add standard or sample to each well used. Incubate at room temperature.

Primary detector antibody



Add prepared biotinylated antibody to each well. Incubate at room temperature.

Streptavidin Label



Add prepared Streptavidin solution. Incubate at room temperature.

Substrate **Colored product**



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|--|----------|--|
| Pre-coated TNF alpha microplate (12 x 8 well strips) | 96 wells | -20°C |
| 20X Wash Buffer Concentrate | 25 mL | -20°C |
| Canine TNF alpha Standard (recombinant) | 2 vials | -20°C |
| Assay Diluent A | 30 mL | -20°C |
| 5X Assay Diluent B | 15 mL | -20°C |
| Biotinylated Canine TNF-alpha detection Antibody (lyophilized) | 2 vials | -20°C |
| 350X HRP-Streptavidin concentrate | 200 µL | -20°C |
| TMB One-Step Substrate Reagent | 12 mL | -20°C |
| Stop Solution | 8 mL | -20°C |

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Completely aspirate all solutions and buffers during wash steps. When preparing your standards, it is critical to briefly spin down

the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.

- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Scientific Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 1X Assay Diluent B

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.2 1X Wash Buffer

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into 380 mL deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 Detection Antibody TNF alpha (biotinylated anti-Canine TNF alpha)

Briefly centrifuge the Detection Antibody vial before use. Add 100 μ L of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly centrifuge the 350X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 350X HRP-Streptavidin concentrate should be diluted 350-fold with 1X Assay Diluent B.

For example: Briefly centrifuge the vial and pipette up and down to mix gently. Add 40 μ L of HRP-Streptavidin concentrate into a tube with 14mL 1X Assay Diluent B to prepare a 1X HRP-Streptavidin solution (do not store the diluted solution). Mix well.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Avoid repeat freezing and thawing.

- 10.1 Briefly centrifuge the vial of canine TNF alpha Standard and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the canine TNF alpha standard vial to prepare a 100 ng/mL **Stock Standard**. Mix thoroughly but gently.
- 10.2 Label tubes #1-8.
- 10.3 Prepare the 700pg/mL **Standard #1** by adding 7µL Stock Standard into tube #1 along with 993 µL Assay Diluent A or 1X Assay Diluent B. Mix thoroughly but gently.
- 10.4 Add 300 µL Assay Diluent A or 1X Assay Diluent B into tubes 2-8.
- 10.5 Prepare **Standard #2** by adding 200 µL Standard #1 to tube #2. Mix thoroughly but gently.
- 10.6 Prepare **Standard #3** by adding 200 µL from Standard #2 to tube #3. Mix thoroughly but gently.
- 10.7 Using the table below as a guide; prepare further serial dilutions.
- 10.8 Standard #8 contains no standard protein and is the Blank control.

Standard Dilution Preparation Table

| Standard # | Sample to Dilute | Volume to Dilute (μL) | Volume of Diluent (μL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|------------|------------------|-----------------------|------------------------|------------------------|---------------------|
| 1 | Stock | 7 | 993 | 100,000 | 700 |
| 2 | Standard #1 | 200 | 300 | 700 | 280 |
| 3 | Standard #2 | 200 | 300 | 280 | 112 |
| 4 | Standard #3 | 200 | 300 | 112 | 44.8 |
| 5 | Standard #4 | 200 | 300 | 44.8 | 17.92 |
| 6 | Standard #5 | 200 | 300 | 17.92 | 7.17 |
| 7 | Standard #6 | 200 | 300 | 7.17 | 2.87 |
| 8 (Blank) | none | - | 300 | 0 | 0 |



11. SAMPLE PREPARATION

- Plasma sample should be collected using EDTA or citrate as an anticoagulant. Heparin is not recommended for use in this assay. Sample Dilution: If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent B should be used for dilution of cell culture supernatants.
- Suggested dilution for normal serum/plasma: 2 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator and fall within the values of the standard curve for accurate quantification.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**

It is recommended to assay all standards, controls and samples in duplicate.

- 13.1. Add 100 μ L of each standard (see Standard Preparations, section or sample into appropriate wells. Cover plate and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
- 13.2. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 μ L 1X Wash Buffer using a multi-channel pipette or automatic plate washer. Complete aspiration of liquid at each step is recommended for best results. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it by tapping gently against clean paper towels.
- 13.3. Add 100 μ L of the prepared Detection Antibody TNF alpha (see Reagent Preparation section) to each well. Incubate for 1 hour at room temperature with gentle shaking.
- 13.4. Discard the solution. Repeat the wash as in step 13.2.
- 13.5. Add 100 μ L of prepared 1X HRP-Streptavidin solution (see Reagent Preparation section) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
- 13.6. Discard the solution. Repeat the wash as in step 13.2.
- 13.7. Add 100 μ L of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
- 13.8. Add 50 μ L of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average Blank absorbance. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Do not force the best fit line through the origin.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

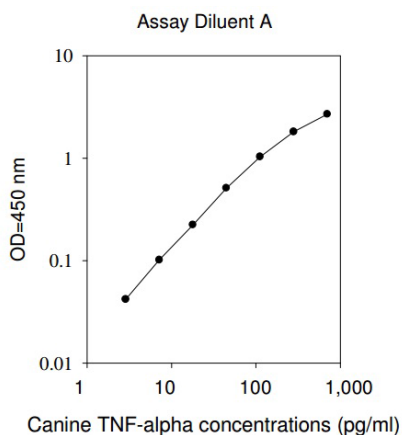


Figure 1. Example of typical canine TNF alpha standard curve using Assay Diluent A (used for serum/plasma standards). The standard curve was prepared as described in Section 10.

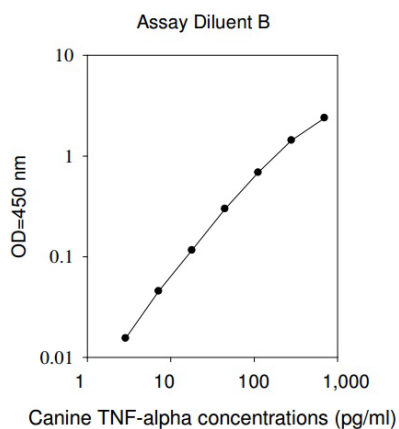


Figure 2. Example of typical canine TNF alpha standard curve using Assay Diluent B (used for cell culture supernatants). The standard curve was prepared as described in Section 10.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of TNF alpha is 2 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of canine TNF alpha into canine serum, plasma and cell culture media. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|--------------------|--------------------|-----------|
| Serum | 101.1 | 93-109 |
| Plasma | 97.84 | 91-104 |
| Cell culture media | 118.4 | 110-126 |

LINEARITY OF DILUTION -

| Serum Dilution | Average % Expected Value | Range (%) |
|----------------|--------------------------|-----------|
| 1:2 | 112.8 | 105-121 |
| 1:4 | 113.3 | 105-121 |

| Plasma Dilution | Average % Expected Value | Range (%) |
|-----------------|--------------------------|-----------|
| 1:2 | 115.7 | 108-124 |
| 1:4 | 118.4 | 110-126 |

| Cell Culture Media Dilution | Average % Expected Value | Range (%) |
|-----------------------------|--------------------------|-----------|
| 1:2 | 99.98 | 92-108 |
| 1:4 | 93.01 | 85-101 |

PRECISION –

| | Intra- Assay | Inter- Assay |
|-----|-------------------------|-------------------------|
| %CV | <10 | <12 |

17. ASSAY SPECIFICITY

The antibodies used within this ELISA kit detect Canine TNF alpha.

The reaction of these antibodies with other species has not been tested.

Please contact our Scientific Support team for more information.

18. TROUBLESHOOTING

| Problem | Cause | Solution |
|---------------------|---|---|
| Poor standard curve | Inaccurate pipetting | Check pipette performance |
| | Improper standards dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation time; change to overnight standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipette performance and ensure correct preparation |
| High %CV | Inaccurate pipetting | Check pipette performance |
| High background | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, ensure it is working properly. |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light. |
| | Stop solution | Stop solution should be added to each well before measuring |

19. NOTES

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