



## **PRODUCT INFORMATION & ELISA MANUAL**

### **TNF-alpha Antibody Pair [HRP] *NBP2-79390***

***Sample Insert for reference use only***

Matched Antibody Pair utilized in an Enzyme-linked  
Immunosorbent Assay for quantitative detection of  
Ferret TNF-alpha.

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

## **BACKGROUND**

Tumor necrosis factor alpha (TNF-alpha), also known as TNF, TNFA or TNFSF2, is the prototypic cytokine of the TNF superfamily, and is a multifunctional molecule involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Two receptors, TNF-R1 (TNF receptor type 1; CD120a; p55/60) and TNF-R2 (TNF receptor type 2; CD120b; p75/80), bind to TNF-alpha. TNF-alpha protein is produced mainly by macrophages, and large amounts of this cytokine are released in response to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1). TNF-alpha is involved in fighting against the tumorigenesis, thus, is regarded as a molecular insight in cancer treatment.

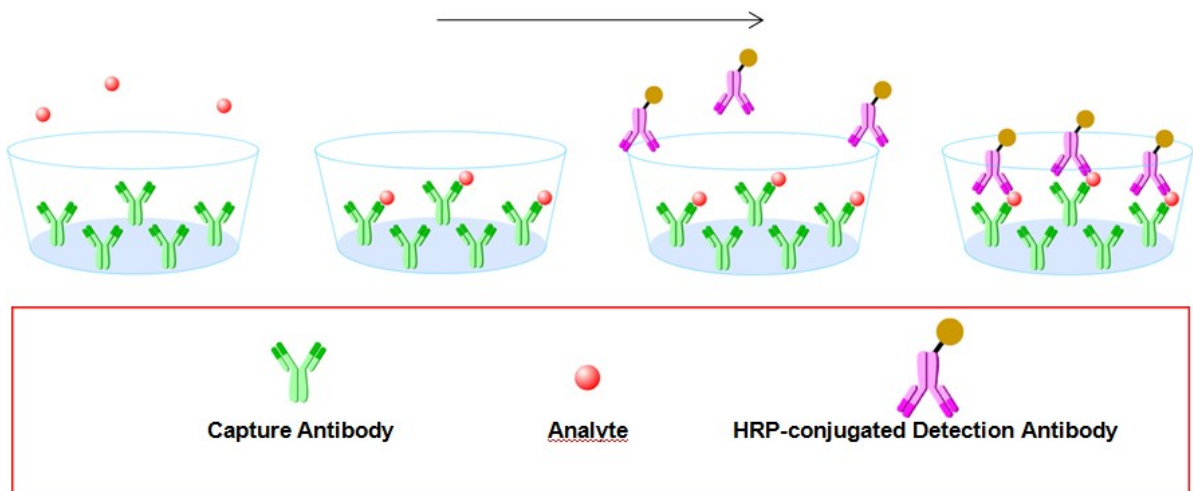
## PRINCIPLE OF THE TEST

The Novus Biologicals TNF-alpha Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for TNF-alpha coated on a 96-well plate. Standards and samples are added to the wells, and any TNF-alpha present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-TNF- alpha / TNFA monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of TNF-alpha present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

## INTENDED USE

- ◆ The Ferret TNF-alpha Antibody Pair [HRP] is for the quantitative determination of Ferret TNF-alpha.
- ◆ This TNF-alpha Antibody Pair [HRP] contains the basic components required for the development of sandwich ELISAs.

## ASSAY PROCEDURE SUMMARY



**This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.**

## MATERIALS PROVIDED

**Bring all reagents to room temperature before use.**

**Capture Antibody** – 05 mg/mL of rabbit anti-ferret TNF-alpha monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

**Detection Antibody** – 0.2 mg/mL mouse anti-ferret TNF-alpha monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.

**Standard** – Each vial contains 70 ng of recombinant ferret TNF-alpha. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A six-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 5 ng/mL is recommended.

## SOLUTIONS REQUIRED

**PBS** - 136.9 mM NaCl, 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.7 mM KCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 0.2 µm filtered

**TBS** - 20 mM Tris, 150 mM NaCl, pH 7.4

**Wash Buffer** - 0.05% Tween20 in TBS, pH 7.2 - 7.4

**Blocking Buffer** - 2% BSA in Wash Buffer

**Sample dilution buffer** - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Detection antibody dilution buffer** - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Substrate Solution** : To achieve best assay results, fresh substrate solution is recommended

**Substrate stock solution** - 10mg / ml TMB ( Tetramethylbenzidine ) in DMSO

**Substrate dilution buffer** - 0.05M Na<sub>2</sub>HPO<sub>4</sub> and 0.025M citric acid ; adjust pH to 5.5

**Substrate working solution** - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H<sub>2</sub>O<sub>2</sub> , mix it well

**Stop Solution** - 2 N H<sub>2</sub>SO<sub>4</sub>

## PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## STORAGE

**Capture Antibody:** Aliquot and store at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

**Detection Antibody:** Store at  $4^{\circ}\text{C}$  and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

**Standard:** Store lyophilized standard at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at  $-80^{\circ}\text{C}$  for up to 1 month. Avoid repeated freeze-thaw cycles.

## GENERAL ELISA PROTOCOL

### Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100 $\mu$ L per well of the diluted capture antibody. Seal the plate and incubate overnight at 4 $^{\circ}$ C.
2. Aspirate each well and wash with at least 300 $\mu$ l wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300  $\mu$ L of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

### Assay Procedure

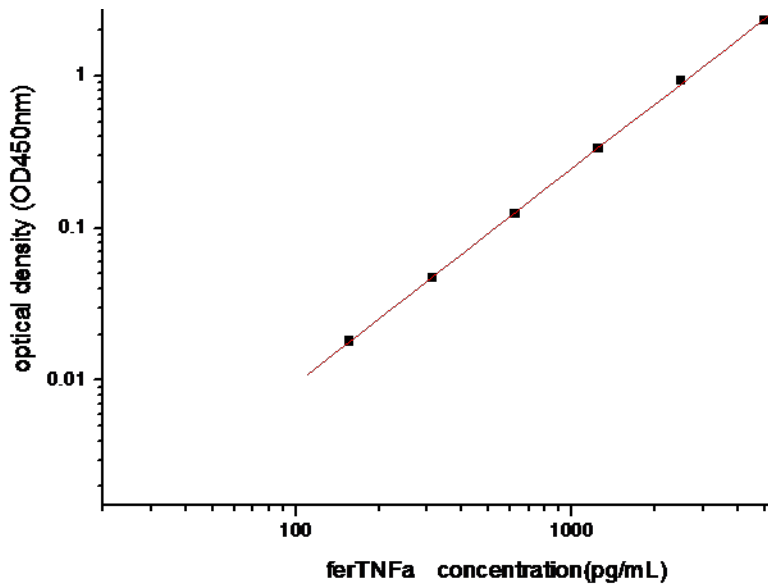
1. Add 100  $\mu$ L of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100  $\mu$ L of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200  $\mu$ L of substrate solution to each well. Incubate for 20 minutes at room temperature ( **if substrate solution is not as requested, the incubation time should be optimized** ). Avoid placing the plate in direct light.
6. Add 50  $\mu$ L of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

## TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



| Concentration (pg/ml) | Zero standard subtracted OD |
|-----------------------|-----------------------------|
| <b>0</b>              | <b>0.000</b>                |
| <b>156.25</b>         | <b>0.018</b>                |
| <b>312.5</b>          | <b>0.047</b>                |
| <b>625</b>            | <b>0.124</b>                |
| <b>1250</b>           | <b>0.333</b>                |
| <b>2500</b>           | <b>0.931</b>                |
| <b>5000</b>           | <b>2.327</b>                |

## PERFORMANCE CHARACTERISTIC

### SENSITIVITY

The minimum detectable dose of Ferret TNF-alpha was determined to be approximately 156.25 pg/mL. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

## TROUBLE SHOOTING

| Problems             | Possible Sources  | Solutions  |
|----------------------|---|--|
| No signal            | Incorrect or no Detection Antibody was added                          | Add appropriate Detection Antibody and continue                                      |
|                      | Substrate solution was not added                                      | Add substrate solution and continue  |
|                      | Incorrect storage condition   | Check if the kit is stored at recommended condition and used before expiration date  |
| Poor Standard Curve  | Standard was incompletely reconstituted or was inappropriately stored | Aliquot reconstituted standard and store at -80 °C                                   |
|                      | Imprecise / inaccurate pipetting                                      | Check / calibrate pipettes   |
|                      | Incubations done at inappropriate temperature, timing or agitation    | Follow the general ELISA protocol  |
|                      | Background wells were contaminated                                    | Avoid cross contamination by using the sealer appropriately                          |
| Poor detection value | The concentration of antigen in samples was too low                   | Enriching samples to increase the concentration of antigen                           |
|                      | Samples were ineffective  | Check if the samples are stored at cold environment. Detect samples in timely manner |
| High Background      | Insufficient washes   | Use multichannel pipettes without touching the reagents on the plate                 |
|                      |   | Increase cycles of washes and soaking time between washes                            |
|                      | TMB Substrate Solution was contaminated                               | TMB Substrate Solution should be clear and colorless prior to addition to wells      |
|                      | Materials were contaminated.  | Use clean plates, tubes and pipettes tips  |
| Non-specificity      | Samples were contaminated   | Avoid cross contamination of samples   |
|                      | The concentration of samples was too high                             | Try higher dilution rate of samples  |





# **Ferret TNF-alpha Antibody Pair [HRP]**

## **Notes**