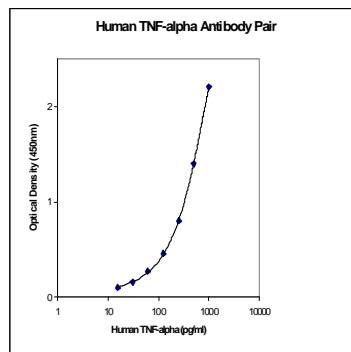


Catalog # CHC1753

1. **Coating Antibody:** **Anti-Human TNF- $\alpha$  (0.250mg/0.125mL)**  
 Part Number: 58.175.09  
 Lot Number: **8C10/1**  
 Form: Liquid, 1 vial, contains 0.1% sodium azide  
 Storage: Store at 2-8°C until expiration date.  
 Recommended Dilution: Dilute to 2  $\mu$ g/mL with Coating Buffer A (Cat. # CB07100, or see Recommended Buffers). For example, to make 10 mL (enough to coat 1 plate), add 10  $\mu$ L coating antibody to 9.990 mL Coating Buffer A.
  
2. **Detection Antibody:** **Anti-Human TNF- $\alpha$  Biotin (0.025mg/0.125mL)**  
 Part Number: 58.175.03  
 Lot Number: **8C11/1**  
 Form: Liquid, 1 vial, contains 0.1% sodium azide  
 Storage: Store at 2-8°C until expiration date.  
 Recommended Dilution: Dilute to 0.32  $\mu$ g/mL with Assay Buffer (Cat. # DS98200, or see Recommended Buffers). For example, to make enough for 1 plate, add 8.8  $\mu$ L detection antibody to 5.4912 mL Assay Buffer.
  
3. **Standard:** **Recombinant Human TNF- $\alpha$**   
 Part Number: 58.175.10 (additional vials of standard may be purchased using this part number)  
 Lot Number: **6B10/2**  
 Form: Lyophilized, 3 vials  
 Storage: Store at 2-8°C.  
 Reconstitution: Reconstitute with Assay Buffer (Cat. # DS98200 or see Recommended Buffers) to yield a stock of 10,000 pg/mL. After 10 minutes of rehydration, use the standard stock immediately or aliquot in polypropylene tubes and freeze at -80°C. *Do not store at room temperature or at 4°C and do not subject to more than one freeze-thaw cycle.*  
 Standard Curve: Dilute standard stock to 1,000 pg/mL (100  $\mu$ L stock plus 900  $\mu$ L Assay Buffer) with Assay Buffer (Cat. # DS98200 or see Recommended Buffers). Add 300  $\mu$ L Assay Buffer to 6 tubes and label as 500, 250, 125, 62.5, 31.25, and 15.6 pg/mL. Make serial dilutions starting with 1,000 pg/mL by transferring 300  $\mu$ L of each standard to next tube and vortexing each tube. Assay Buffer should be used as the zero standard.
  
4. **Streptavidin-HRP:** **0.25mL**  
 Part Number: 41.000.03  
 Lot Number: **7K5/1**  
 Form: Liquid, 1 vial, contains 0.05% thymol  
 Storage: Store at 2-8°C until expiration date.  
 Recommended Dilution: Dilute to 1/625 For example, to make enough for 1 plate, add 16  $\mu$ L of streptavidin-HRP to 9.984 mL of Assay Buffer (Cat. # DS98200 or see Recommended Buffers).



Representative standard curve was generated by following the recommended assay procedure, which includes the use of the **Invitrogen Antibody Pair Set (Cat. # CNB0011)**

**For research use only. Caution: Not intended for human or animal therapeutic or diagnostic use.**

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## Intended Use and Materials Provided

The Antibody Pair for Human TNF- $\alpha$  contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of TNF- $\alpha$ . Sufficient quantities of all reagents are provided to yield 10 plates of 96 wells if the recommended assay procedure and recommended storage and handling of materials are followed as specified on this insert. The materials provided are **FOR RESEARCH USE ONLY**.

## Recommended Buffers and Solutions

*The Invitrogen Antibody Pair Buffer Set (Cat. # CNB0011) containing Coating Buffers A and B, Assay Buffer, Substrate Solution (TMB), Stop Solution, and Wash Buffer is recommended.*

- Coating Buffer A:** *Coating Buffer A (Cat. # CB07100) from Invitrogen is recommended. Alternate buffer choice listed below.*  
8.0 g NaCl, 1.13 g Na<sub>2</sub>HPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 0.2 g KCl, 0.1% ProClin™; q.s. to 1.0 L with distilled H<sub>2</sub>O, pH to 7.4.
- Coating Buffer B:** *Coating Buffer B (Cat. # CB01100) from Invitrogen is recommended. Alternate buffer choice listed below.*  
4.3 g NaHCO<sub>3</sub>, 5.3 g Na<sub>2</sub>CO<sub>3</sub>, 0.1% ProClin™; q.s. to 1.0 L with distilled H<sub>2</sub>O, pH to 9.4.
- Assay Buffer:** *Assay Buffer (Cat. # DS98200) from Invitrogen is recommended. Alternate buffer choice listed below.*  
8.0 g NaCl, 1.13 g Na<sub>2</sub>HPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 0.2 g KCl, 5.0 g bovine serum albumin (fraction V), 1 mL Tween 20 and 0.5% ProClin™ as a preservative; q.s. to 1.0 L with distilled H<sub>2</sub>O, pH to 7.4.
- Wash Buffer:** *Wash Buffer 25X (Cat. # WB01) from Invitrogen is recommended. Alternate buffer choice listed below.*  
0.2 g KH<sub>2</sub>PO<sub>4</sub>, 1.9 g, K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.4 g EDTA, 0.5 mL Tween 20; q.s. to 1.0 L with distilled H<sub>2</sub>O, pH to 7.4.
- Substrate Solution:** *TMB (Cat. # SB01) from Invitrogen is recommended. Alternate solution choice listed below.*  
Tetramethylbenzidine (TMB) and Hydrogen Peroxide.
- Stop Solution:** *Stop Solution (Cat.# SS01100) from Invitrogen is recommended. Alternate solution choice listed below.*  
1.8 N H<sub>2</sub>SO<sub>4</sub>.

## Assay Optimization

Antibody Pairs from Invitrogen are designed to be very flexible for your experiments. Consequently, the assay procedure contains only recommendations. The assay procedure has been optimized for use with tissue culture samples. However, serum and plasma samples may be used but may require that certain assay parameters be modified. Investigators are advised to determine optimal buffer formulations, concentrations and incubation times for individual applications.

## Recommended Assay Procedure

- Prepare coating solution by diluting the coating antibody. See “coating antibody” section for the recommended coating antibody dilution.
- Coat plates with 100  $\mu$ L per well of the coating solution. Cover plates and incubate overnight (12-18 hr.) at 4°C.
- Aspirate wells and wash 1 time with > 400  $\mu$ L of Wash Buffer per well. Following wash, invert and tap on absorbent paper to remove excess liquid.
- Block plate with 300  $\mu$ L per well of Assay Buffer for 1 hour at room temperature.
- Aspirate, invert, and tap on absorbent paper to remove excess liquid.
- Prepare standards and sample dilutions in Assay Buffer (or in a diluent that most closely matches the matrix of your sample). For recommended dilutions and storage of the standard, see “standard” section.
- Pipette 100  $\mu$ L of standards (in duplicate), samples into designated wells.
- Immediately following step 7, add 50  $\mu$ L of the working detection antibody into each well. For recommended dilutions, see “detection antibody” section. **Incubate for 2 hours at room temperature with continual shaking (700 rpm).**
- Aspirate and wash 5 times using the method in step 3.
- Add 100  $\mu$ L of the working streptavidin-HRP solution into each well. For recommended dilutions, see “streptavidin-HRP conjugate” section. **Incubate for 30 minutes at room temperature with continual shaking (700 rpm).**
- Aspirate and wash 5 times using the method in step 3.
- Add 100  $\mu$ L of the TMB substrate to each well. Incubate plate for 30 minutes at room temperature with continual shaking (700 rpm).
- Add 100  $\mu$ L of Stop Solution to each well.
- Measure absorbance at 450 nm (reference absorbance: 650 nm) within 30 minutes of adding Stop Solution. Calculate results using a log-log or 4-parameter curve fit.

## Additional Materials Required

- 96 well NUNC MaxiSorp microplates; NUNC Cat. # 434797.
- Pipettes, plate shaker and timer.
- Microplate reader with a detector that can measure absorbance at 450 nm.
- 1 L graduated cylinder; plate washer or wash bottle.
- Polypropylene tubes for standards and sample dilutions, if needed.

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