

## PRODUCT INFORMATION SUMMARY

### Human TNF Alpha ELISA Construction Kit

Product Number RHF550CK  
Approx. 960 tests

Product Number **RHF550CKC**  
**With Developing Reagents:**

Capture Antibody	<b>100.0 ug</b>	ELISA Coating Stabilizer	50 mL
Biotin Tracer	25.0 ug or 0.5 mL	Streptavidin-HRP	0.5 mL
Antigen Standard	1.0 ug or 10.0 ug	TMB Substrate ( 50 mL x 2)	
		WASH Buffer (20X)	100 mL

**RHF550CKP** - with Developing Reagents and ten plates

#### DESCRIPTION:

Human soluble TNF Alpha ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard for development of **approximately** ten microplates. Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

#### CAPTURE ANTIBODY:

Provided as lyophilized, **100 ug**, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL).

#### TRACER ANTIBODY:

Provided as 25 ug ( 0.5 mL Liquid or lyophilized - **see vial**) of Biotin labeled, antigen-affinity purified antibody, additive-free. For lyophilized : Reconstitute in 500 uL sterile water **containing 0.1% BSA.** ( FREEZE aliquots for long-term storage).

For vial provided as **Liquid - Store refrigerated only - contains preservative**

**STANDARD:** Provided as 1.0 ug or 10.0 ug ( see vial ) of recombinant Human TNF Alpha. Quick-spin and reconstitute in 100 uL of sterile water (pH 7.2). **Allow reconstituted standard to sit for 2 hours at room temperature before use.** Further dilutions can be made in 0.1% BSA in PBS.

**DEVELOPING REAGENTS:** Supplied with catalog # ending in "CKC".

- ELISA Coating/ Blocking Reagent ( EA150C) 50.0 mL ( 5X Solution)
- Streptavidin-HRP ( S100180C) 0.5 mL - store @ -20 Deg. C.
- TMB Substrate Solutions - Part A and Part B ( 50.0 mL each)  
cat # ES200C
- WASH Buffer (20X) - Dilute 1 part with 19 parts distilled water

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Antibodies (Capture and Tracer) can be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

For **biotin** tracer antibody Provided as 0.5 mL, STORE refrigerated only - contains preservative.

Standard ( rec. TNF Alpha) can be stored in liquid state ( @ 4 Deg. C.) For up to one week, or store **frozen, with addition of 0.1% BSA**, at -20 Deg. C. for up to 2 months. AVOID repeat freeze-thaw.

**MATERIALS RECOMMENDED:**

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404  
Tween -20.  
BSA  
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar  
TMB Substrate : ANTIGENIX cat # **ES200**  
Dubelco's PBS (10X)  
ANTIGENIX **ELISA Coating Stabilizer** ( cat no: **EA150**)

**RECOMMENDED SOLUTIONS:**

See **ANTIGENIX Developing reagents above.**

PBS: Dilute to 1XPBS in sterile water  
WASH BUFFER: ANTIGENIX WB200 or 0.05% Tween-20 in PBS.  
BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer - recommended- (EA150) or 1% BSA in PBS**  
Substrate Solution: TMB Substrate Solution ( cat # **ES200**)  
Diluent: 0.1% BSA in PBS  
2N Sulfuric acid ( stop solution)

**PLATE PREPARATION:**

1. **Dilute portion** of capture antibody with 0.05M Carbonate buffer ( or PBS ) to concentration **1.0 ug/mL.**

Immediately **add** 100 uL to each ELISA well. Seal the plate and incubate overnight at room temperature.

2. Aspirate wells to remove all liquid and wash **4 times** using 300 uL of wash buffer per well. After last wash, add 200 uL ANTIGENIX AMERICA **ELISA coating stabilizer (cat # EA150)** and incubate for 60 minutes at room temperature. ( With coating stabilizer, DO NOT let plate dry prior to use of coating stabilizer. This will **stabilize and Block in one step!** Refer to data sheet EA150 for complete description of use.
3. With ANTIGENIX coating stabilizer ( **recommended** ) aspirate plate but **DO NOT WASH.** For extended storage- dry plate in humidity controlled chamber or similar. ( see data sheet EA150). With standard block reagent, aspirate plate and wash 3X with 300 uL wash buffer.

**PROTOCOL:**

**STANDARD/SAMPLE:** Dilute **a portion of the** standard ( store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from **5.0 ng/mL** to zero in diluent (8 point, 1:2 serial dilution). Immediately **add** 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for approx. 2 hours.

**DETECTION:** Aspirate and wash plate 4 times. **Dilute** portion of detection (Tracer) antibody in diluent to concentration of **0.20 ug/mL**. ( approx. 1:250 from 50.0 ug/mL). **Add** 100 uL per well. **Incubate** at room temperature for approx. 1 hour. Note: detection antibody can be used in approximate range of 0.10 - 0.50 ug/mL, you may need to optimize for subsequent plates.

**STREPTAVIDIN-HRP:** Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** in diluent ( follow recommended dilution of manufacturer). (May need to optimize) **Add** 100 uL per well, incubate 30 minutes at room temperature.

**SUBSTRATE:** Aspirate and wash plate 4 times. **Add** 100 uL substrate solution to each well (follow directions for substrate preparation and handling from manufacturer's data sheet). Incubate at room temp. for color development. **Stop** the color reaction after 10 - 15 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, read plate @ 450 nm within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.25 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

**RESEARCH USE ONLY -NOT For DIAGNOSTIC USE**

**NOTE:** Kit can be ordered with developing reagents ( cat# ends in 'CKC') -see page 1.

**X-REACTIVITY Data:**

**No** X-reactivity was observed @ 40-50 ng/mL with following factors:

Rat TNF alpha  
Mouse TNF alpha; sRANKL;  
Human: TNF beta; TNFR1 and R2; TRAIL; TWEAK; TWEAK-R; VEGF;  
BAFF; sFasL; CD40L