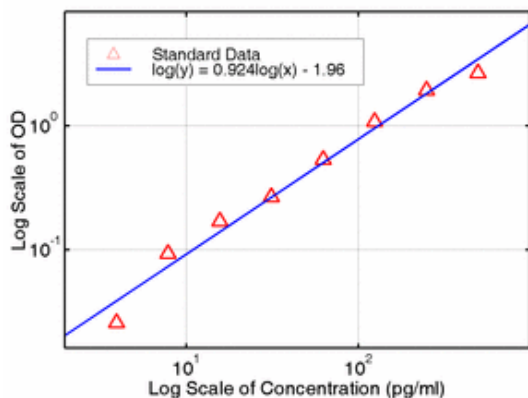


## Human TNF alpha ELISA Ready-SET-Go!<sup>®</sup>

Catalog Number: 88-7346

Also Known As: Tumor Necrosis Factor alpha, TNFa TNF-a

RUO: For Research Use Only



Standard curve of Human TN $\alpha$  ELISA Ready-SET-Go!<sup>®</sup>


### Product Information

Contents: Human TNF alpha ELISA Ready-SET-Go!<sup>®</sup>

**REF** Catalog Number: 88-7346


Sensitivity: 4 pg/ml

Standard Curve Range: 4 - 500 pg/ml

 Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.

**LOT** Batch Code: Refer to Vial

 Use By: Refer to Vial

 Caution, contains Azide

### Description

This Human TNF- $\alpha$  ELISA Ready-SET-Go! reagent set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of human TNF- $\alpha$  protein levels from samples including serum, plasma, and supernatants from cell cultures.

### Components

Capture Antibody. Pre-titrated, purified antibody

Detection Antibody. Pre-titrated, biotin-conjugated antibody

Standard. Recombinant cytokine for generating standard curve and calibrating samples

ELISA/ELISPOT Coating Buffer Powder. This Ready-Set-Go! ELISA Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH2O and filter (0.22  $\mu$ M)) or 10X PBS ELISA Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH2O).

Assay Diluent. 5X concentrated

Detection enzyme. Pre-titrated Avidin-HRP

Substrate Solution. Tetramethylbenzidine (TMB) Substrate Solution

Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards

96 Well Plate. Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)

### References

Marseille-Tremblay C, Ethier-Chiasson M, Forest JC, Giguère Y, Masse A, Mounier C, Lafond J. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. *Mol Reprod Dev.* 2008 Jun;75(6):1054-62. (RSG ELISA kit, tissue homogenate, PubMed)

Tversky JR, Le TV, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT. Human blood dendritic cells from allergic subjects have impaired capacity to produce interferon-alpha via Toll-like receptor 9. *Clin Exp Allergy.* 2008 May;38(5):781-8. (RSG ELISA kit, TC supernatant, PubMed)

Ahuja J, Lepoutre V, Wigdahl B, Khan ZK, Jain P. Induction of pro-inflammatory cytokines by human T-cell leukemia virus type-1 Tax protein as determined by multiplexed cytokine protein array analyses of human dendritic cells. *Biomed Pharmacother.* 2007 May;61(4):201-8. (RSG ELISA kit, TC supernatant, PubMed)

Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest*. 2003 Jul;124(1):177-85. (RSG ELISA kit, TC supernatant, serum, and lavage fluid, PubMed)

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Not for further distribution without written consent.

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## TDS Protocol

### Research Use Only

#### Other Materials Needed

- ☐ Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H<sub>3</sub>PO<sub>4</sub> or 2N H<sub>2</sub>SO<sub>4</sub>

☐ Pipettes and pipettors

☐ Refrigerator

☐ 96-well plate (Corning Costar 9018)

**NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.**

☐ 96-well ELISA plate reader (microplate spectrophotometer)

☐ ELISA plate washer

**NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.**

#### Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

#### Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

#### Storage Instructions for Other Set Reagents

Store at 4°C.

#### Time Requirements

- ☐ 1 overnight incubation
- ☐ 4½-hour incubations
- ☐ 1 hour washing and analyzing samples

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## TDS Protocol

### Research Use Only Experimental Procedure

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1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer\*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.\* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using 1X Assay Diluent\*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent\* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP\* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer\* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

**\*NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.**

## TDS Protocol

### Research Use Only Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532

\* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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## TDS Protocol

### Research Use Only

C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each step's incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the "frost free" freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date