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PrecisionBind Human Tumor Necrosis Factor Alpha (TNFa / TNF-alpha / TNFA) ELISA

REF : KB1145

Ver 1.1

RUO

NIBSC Calibrated Assay

*The standard used in the kit is calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. 1 ng of supplied standard equals 46 U of 12/154 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 46 U of TNFα as per NIBSC.

ELISA for Accurate Quantitation of Human TNF-α from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KB1145** Σ **96 tests**

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PrecisionBind Human Tumor Necrosis Factor Alpha (TNFa / TNF-alpha / TNFA) ELISA

Introduction:

Tumor Necrosis Factor-α (TNF-α) is a potent lymphoid factor which exerts cytotoxic effects on a wide range of tumor cells and certain other target cells. Human TNF-α is a 17.4 kDa protein containing 157 amino acid residues.

Long Name: Tumor necrosis factor-α

Entrez Gene IDs: 7124 (Human); 21926 (Mouse); 24835 (Rat); 397086 (Porcine); 280943 (Bovine); 403922 (Canine); 102139631 (Cynomolgus Monkey); 100033834 (Equine); 493755 (Feline); 100009088 (Rabbit)

Alternate Names: APC1 protein, Cachectin, Cachetin, DIF, TNF, TNF, monocyte-derived, TNF-A, TNF-alpha, TNF-alpha cachectin, TNFA, TNFATNF, macrophage-derived, TNFG1F, TNFSF1A, TNFSF2, TNFSF2TNF superfamily, member 2, TNFalpha, Tumor necrosis factor ligand superfamily member 2, tumor necrosis factor, tumor necrosis factor (TNF superfamily, member 2), tumor necrosis factor alpha, tumor necrosis factor-alpha.

Intended Use:

PrecisionBind Human Tumor Necrosis Factor Alpha (TNFa / TNF-alpha / TNFA) ELISA is specifically designed for the accurate quantitation of human TNF-α from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing human TNF alpha react with already coated affinity purified capture anti-human TNF alpha antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. HRP conjugated Anti-human TNF alpha detection antibody is added leading to formation of a sandwich complex of solid phase antibody-human TNF alpha-biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3', 5, 5' Tetra Methyl Benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader at 450 nm and it is directly proportional to the concentration of Human TNF alpha present in the samples.

Materials Provided:

1. Anti-Human TNF-α Antibody Coated Microtiter Plate (12x8 wells) – 1 no
2. Recombinant Human TNF-α Standard (lyophilized) – 2 vials
3. Anti-Human TNF-α HRP Conjugated Detection Antibody – 1 vial
4. Anti-Human TNF-α HRP Conjugated Detection Diluent – 12 ml
5. (1X) Assay Diluent – 50 ml
6. (20X) Wash Buffer– 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Store recombinant **Standard at 2-8°C**. After reconstitution aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
4. Before using, bring all components to room temperature (18-24ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
5. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot-specific instructions for preparation of the reagents mentioned in the Reagent Preparation Sheet. *Note each reagent sheet is specific for a particular Lot only and is not to be interchanged amongst different lots*.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human TNF-α. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human TNF-α present in the sample.
3. Human TNF-α concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human TNF-α.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add 100 ul of Standards and Samples to each well, Seal plate and incubate for 2 hours at RT.

3. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add 100 ul of diluted **Detection Antibody** solution to each well, Seal plate and incubate for 1 hour at RT.

5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3.

6. Add 100 ul of **TMB Substrate** solution and incubate in the dark for 30 minutes at RT. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

7. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

8. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

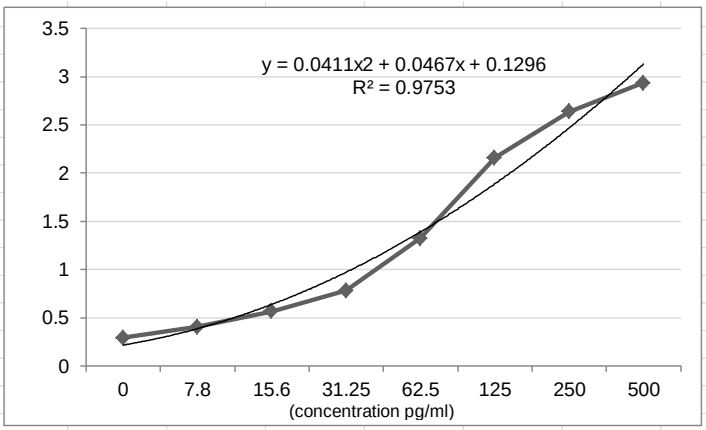
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Data (representative only)

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration (pg/ml)	% Interpolated Concentration against Actual Concentration
0	0.291		--
7.8	0.404	8.3	106.1
15.6	0.567	18.9	121.4
31.25	0.784	30.2	96.5
62.5	1.324	60.7	97.0
125	2.158	128.8	103.0
250	2.639	247.9	99.2
500	2.934	492.5	98.5

Typical Graph (representative only)



Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD. 10 replicates of '0' standards were evaluated and the LOD is **~3.1 pg/ml**.

Limit of Quantitation (LOQ): It is defined as the lowest concentration of an analyte that can be measured with acceptable precision and accuracy, 10 replicates of '0' standards were evaluated and the LOQ is **~9.3 pg/ml**.

IC_{50}: The half-maximal inhibitory concentration (IC_{50}) in a sandwich ELISA measures the concentration of an inhibitor (such as a drug, molecule, or antibody) required to reduce the binding of a target antigen to the capture/detection antibody pair by 50%. The IC_{50} for PrecisionBind Human Tumor Necrosis Factor Alpha (TNFa / TNF-alpha / TNFA) ELISA is ~**173 pg/ml**.

Lower Limit of Quantification: The lowest concentration of an analyte that can be quantitatively measured with acceptable accuracy and precision. 10 replicates of '0' standards were evaluated and the LLOQ is **≤ 9.3 pg/ml.**

Upper Limit of Quantification: The highest concentration of an analyte that can be quantitatively measured with acceptable accuracy and precision in an assay. 10 replicates of '0' standards were evaluated and the ULOQ is **~500 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human TNF-α. The standard used in the kit is calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. 1 ng of supplied standard equals 46 U of 12/154 NIBSC-standard. Please note that the calibration is lot specific.

Cross-Reactivity:
This assay recognizes natural and recombinant human TNF-α. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1alpha	IL-1beta	IL-2	IL-3	IL-4	IL-7	IL-8	IL-10
GM-CSF	IFN alpha	IFN gamma	LIF	MIP-1alpha	MIP-1beta	MCP-1	OSM
RANTES	TGF beta	TNF beta					

Assay Range:
7.8 pg/ml to 500 pg/ml.

Parallelism and Matrix Effect:
Sample Dilution factor – Human Serum, Human Plasma and Human CSF samples have been tested. Sample dilution Factor for all three matrices is 1:50 dilution.

Neat Human Serum, Human Plasma and Human CSF were spiked with 250 pg/ml Human TNF alpha and ELISA assay was run.

Sample	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
Neat CSF samples	2.655	282.1	112.8
Neat Plasma	2.657	283.1	113.2
Neat Human Serum	2.789	372.4	149.0

A) Serum

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.936	444.0	88.8	112.6
1:200 dilution	250	2.645	241.8	96.7	103.4
1:400 dilution	125	2.099	126.5	101.2	98.8
1:800 dilution	62.5	1.309	59.4	95.1	105.2
1:1600 dilution	31.25	0.758	29.1	93.1	107.4
1:3200 dilution	15.6	0.529	16.7	107.3	93.4
1:6400 dilution	7.8	0.381	7.0	90.3	111.0

B) Plasma

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.844	405.3	81.1	123.4
1:200 dilution	250	2.537	222.5	89.0	112.4
1:400 dilution	125	1.944	120.3	96.3	103.9
1:800 dilution	62.5	1.482	79.9	127.9	78.2
1:1600 dilution	31.25	0.783	35.9	114.8	87.1
1:3200 dilution	15.6	0.457	14.2	90.7	110.4
1:6400 dilution	7.8	0.373	5.5	70.3	142.4

C) Cerebrospinal Fluid

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	500	2.917	456.7	91.3	109.5
1:200 dilution	250	2.502	212.8	85.1	117.5
1:400 dilution	125	1.902	115.9	92.7	107.9
1:800 dilution	62.5	1.427	76.0	121.6	82.2
1:1600 dilution	31.25	0.627	26.3	84.1	118.9
1:3200 dilution	15.6	0.494	17.1	109.6	91.4
1:6400 dilution	7.8	0.400	8.8	112.9	88.7

Results:

i. Parallelism is maintained across the 1:100 to 1:6400 dilutions.
ii. % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii. No significant matrix effect observed at higher dilutions.
iv. The PrecisionBind Human TNFalpha ELISA kit was tested for matrix effect on human serum, plasma, CSF and physiological buffer 7.4 to mimic tear fluid samples.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human TNF-α and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
Serum (n=5)	84-107%	87-108%	82-112%
EDTA plasma (n=5)	83-102%	83-115%	83-118%
Heparin plasma (n=5)	83-99%	80-95%	82-93%

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Limitations of Method:

Any diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. The KB1145 PrecisionBind Human Tumor Necrosis Factor Alpha (TNFa / TNF-alpha / TNFA) ELISA is a research use kit only and is not licensed for In-Vitro Diagnostic Use.

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (<0.1 % w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **human body fluids** or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

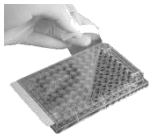
Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN RESULTS

3.  Pipette **100 ul Standards** into respective Standard wells.

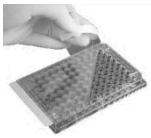
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at room temperature.

6.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

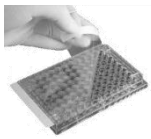
7.  Pipette **100 ul diluted Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at room temperature.

9.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

10.  Pipette **100 ul TMB Substrate** into each wells.

11. Cover plate  and incubate for 

at room temperature.

12.  Pipette **100 ul Stop Solution** into each well.

13. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

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This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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SYMBOLS KEY

MTP	Anti-Human TNF-α Antibody Coated Microtiter Plate (12x8 wells)
STD	Recombinant Human TNF-α Standard, Lyophilized
DETN CONJ	Anti-Human TNF-α HRP Conjugated Detection Antibody
CONJ DETN DIL	Anti-Human TNF-α HRP Conjugated Detection Diluent
1X ASY DIL	(1X) Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature