Human TNF-α ELISA KIT

Catalog Number:

TNA31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

The Eagle Biosciences Human TNF Alpha (TNF-α) ELISA Assay Kit is a solid phase sandwich ELISA for intended for the qualitative and quantitative determination of human TNF Alpha (TNF-α) concentrations in supernatants, buffered solutions, or serum and plasma samples. This assay will recognize both natural and recombinant human TNF Alpha. The TNF Alpha (TNF-α) ELISA Assay Kit is for research use only and not to be used for diagnostic procedures.

INTRODUCTION

Tumor Necrosis Factor (TNFα), also known as cachectin, is a polypeptide cytokine produced by monocytes and macrophages. It functions as a multipotent modulator of immune response and further acts as a potent pyrogen (4, 17). TNFα circulates throughout the body responding to stimuli (infectious agents or tissue injury), activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting. TNFα also inhibits lipoprotein lipase activity resulting in cachexia, a physical wasting condition (4, 17). Activation of B-cells by the Epstein Barr virus can be inhibited by TNFα (15). Due to its varied actions throughout the immune system, TNFα may play a role in the pathogenesis of many disease states. TNFα production is mediated by the action of lymphokines and endotoxins on the macrophage. Purified monocytes produce TNFα within four hours of stimulation by recombinant IL-2 (9) and there is some in vitro evidence to suggest that TNFα is expressed at high levels and with prolonged kinetics in T cells stimulated by both CD2 and CD28 (5). Secretion of TNFα is enhanced by gamma interferon. TNFα then induces or enhances the specific production of Class I MHC antigen, GM-CSF, and IL-1. Recent evidence has suggested an intracellular role for this peptide (23).

TNFα may play a significant role in the pathogenesis of inflammatory disease of the joints and other tissues. Chin et al. (6) found that TNFα, along with gamma interferon and IL-1 increased cell surface expression of ICAM-1 on synovial fibroblasts. Alvaro-Garcia et al. (3) reported that TNFα stimulates synovial proliferation.

Waage et al. (25) found that increased levels of TNFα in patients with septicemia and meningococcal disease correlated with fatal outcome. Scuderi et al. (22) suggest that increased levels of this cytokine may play a role in the host defense mechanism against parasitic infections. Girardin et al. (12) reported that increased serum TNFα levels correlated with the number of risk factors involved in children with gram-negative sepsis and purpura fulminians. Elevated levels of TNFα were also found in individuals suffering from myocarditis (11).

Role for TNFα in the pathogenesis of AIDS has also been pointed out. Alveolar macrophages (AM) from HIV positive individuals with opportunistic lung infections have been shown to spontaneously produce higher levels of TNFα in vitro than those HIV positive individuals without infection and HIV negative controls (14, 16). Krishnan et al. (16) report that higher TNFα production by AM was associated with lower counts of pneumocystis carinii in broncheoalveolar lavage fluid, indicating that TNFα may play a role in the control of this infection in AIDS. IsraelBiet et al. (14) also reported in in vitro studies, that AM that express HIV (p24+) released significantly higher levels of TNFα than p24- alveolar macrophages and controls. Reddy et al. (20) found persistently elevated levels of circulating TNF in HIV seropositive individuals and suggest a possible involvement of this cytokine in the development of AIDS.

Measurement of TNFα levels has also been shown to be useful in transplant research, where Maury et al. (18) and McLaughlin et al. (19). Both reported TNFα to be markedly elevated in renal allograft rejection episodes. Recent evidence has been presented on increased TNFα levels in Bone Marrow Transplant (BMT) (13, 21). BMT patients with major transplant related complications such as interstitial pneumonitis and severe acute graft-versus - host disease had TNFα levels significantly increase over controls (13).

PRINCIPLE OF THE ASSAY

A capture Antibody highly specific for TNFα has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of TNFα samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti- TNFα secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of TNFα present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of TNFα in any sample tested.

REAGENTS

Reagents (Store at 2-8C)	Quantity	Reconstitution
Anti-TNFα Coated plate	1	Ready-to-use (96-well strip pre-coated plate)
Plastic plate covers	2	n/a
TNFα Standard: 800 pg/ml	2	Reconstitute as directed on the vial (see reagent preparation)
Standard Diluent (Buffer)	1 (15mL)	10x Concentrate, dilute in distilled water (see reagent preparation)
Standard Diluent (Serum)	1 (7mL)	Ready-to-use
TNFα Control	2	Reconstitute as directed on the vial (see reagent preparation)
Biotinylated anti-TNFα	1 (0.4mL)	Dilute in biotinylated antibody diluent (see reagent preparation)
Biotinylated Antibody Diluent	1 (7mL)	Ready-to-use
Streptavidin-HRP	2 (5μL)	Add 0.5ml of Streptavidin-HRP diluent prior to use (see reagent preparation)
Streptavidin-HRP Diluent	1 (12mL)	Ready-to-use
Wash Buffer	1 (10mL)	200x concentrate, dilute in distilled water (see reagent preparation)

TMB Substrate	1 (11mL)	Ready-to-use
H_2SO_4 Stop Reagent	1 (11mL)	Ready-to-use

THE REQUIRED ITEMS (not provided):

1. Microplate reader with appropriate filters (450nm required with optional 620nm reference filter)
2. Microplate washer or wash bottle
3. 10, 50, 100, 200 and 1000 µL adjustable single channel micropipettes with disposable tips
4. 50-300µL multi-channel micropipette with disposable tips
5. Multi-channel micropipette reagent reservoirs
6. Distilled water
7. Vortex mixer
8. Miscellaneous laboratory plastic and/or glass, if possible sterile

STORAGE INSTRUCTIONS

Store kit reagents between 2-8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Wash Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.
Standard Diluent Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.
Reconstituted Standard/Control: Once prepared use immediately and do not store
Diluted Biotinylated Anti-TNFα: Once prepared use immediately and do not store
Diluted Streptavidin-HRP: Once prepared use immediately and do not store

SAMPLE COLLECTION, PROCESSING, AND STORAGE

Cell culture supernatants, human serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

1. **Cell Culture Supernates** - Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.
2. **Serum** – Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.
3. **Plasma** – EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma
4. **Storage**—if not analyzed shortly after collection, samples should be aliquoted (250-500µL) to avoid repeated freeze-thaw cycles and stored at -70°C. Avoid multiple freeze-thaw cycles of frozen specimens.
5. **Recommendations**—Do not thaw by heating at 37°C or 56°C. That at room temperature and make sure that sample is completely thawed and homogenous before use. When possible

avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

SAFETY & PRECAUTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance's within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min of the washing of the microtiter plate.

ASSAY DESIGN

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard, zero anda control should be tested in duplicate. Remove sufficient microwell strips for testing from the pouch immediately prior to use. Return any unused wells not required for this assay with desiccant to the pouch. Seal tightly and reture to 2-8oC storage.

Example Plate Layout: (example shown for a 6 point standard curve)

	Standards /Controls		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	800	800										
B	400	400										
C	200	200										
D	100	100										
E	50	50										
F	25	25										
G	0	0										
H	Ctrl	Ctrl										

All remaining empty wells can be used to test samples in duplicate.

REAGENT PREPARATION

Bring all reagents of the Human TNF Alpha (TNF-α) ELISA Assay Kit to room temperature before use.

1. **Wash Buffer—**If crystals have formed in the concentrate Wash Buffer, warm it gently until complete dissolution. Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2˚-25˚C.

2. **Standard Diluent (Buffer)**- If crystals have formed in the concentrate Standard Diluent, warm it gently until complete dissolution. Dilute the (10X) concentrate Standard Dilutent 10 fold with distilled water to give a 1X working solution. Pour entire contents of the concentrate Standard Diluent into a clea appropriate graduated cylinder. Bring final volume with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2-25°C. Please see example volumes below:

Standard Diluent concentrate (mL)	Distilled Water (mL)
15	135
25	225

3. **Standard-** Depending on the type of samples you are assaying, the kit may include two standard diluents. Because biological fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure, you should reconstitute standard vials with the most appropriate Standard Diluent.

   For **serum and plasma samples:** use Standard Diluent - Serum.
   For **cell culture supernatants**: use Standard Diluent Buffer 1X

   Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 800pg/ml of TNFα.

Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 800 to 25 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200µL of the reconstituted standard to well's A1 and A2, which provides the highest concentration standard at 800pg/ml.
- Add 100µL of appropriate standard diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100µL from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 800pg/ml to 25pg/ml.
- Discard 100µl from the final wells of the standard curve (F1 and F2).

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

5. **Preparation of controls -** Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to your samples.

   For **serum and plasma samples**: use Standard Diluent - Serum.

   For **cells culture supernatants:** use Standard Diluent Buffer 1X

   The supplied Controls must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution at the concentration stated on the vial. Do not store after use.

6. **Preparation of Biotinylated anti-TNFα**- It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-TNFα with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of Wells	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

7. **Preparation of Steptavidin-HRP-** it is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

   Dilute the 5µl vial with 0.5ml of HRP diluent immediately before use. Do not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of Wells	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

METHOD

We strongly recommend that every vial is mixed without foaming prior to use.

Prepare all reagents as shown above.

Note: final preparation of Biotinylated Secondary Antibody and Streptavidin-HRP should occur immediately before use.

1. **Prepare standard curve** as shown in 'Preparation of Standards' section above and add in duplicate to appropriate wells.
2. Add 100µl of each **Sample, Standard, Control and zero (appropriate standard diluent)** in duplicate to appropriate number of wells.
3. Add 50µl of diluted **biotinylated anti-TNFα** to all wells
4. Cover with a plastic plate cover and incubate at room temperature (18-25°C) for **3 hours**
5. Remove the cover and wash the plate as follows;
   a) Aspirate the liquid from each well
   b) Dispense 0.3 ml of **1x Wash buffer** into each well
   c) Aspirate the contents of each well
   d) Repeat step b and c another 2 times
6. Add 100µl of **Streptavidin-HRP** solution into all wells
7. Cover with a plastic plate cover and incubate at room temperature (18-25°C) for **30 min**.
8. Repeat wash step 5
9. Add 100µl of ready-to-use **TMB substrate solution** into all wells
10. Incubate in the dark for **12-15 minutes** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.
11. Add 100µl of **H_2SO_4; Stop Reagent** into all wells.
12. Read the absorbance value of each well (immediately after step 11) on a spectrophotometer using 450nm as the primary wavelength and optionally 620nm as the reference wavelength (610nm-650nm is acceptable)

*Incubation time of the TMB substrate is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the color

development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.

DATA ANALYSIS

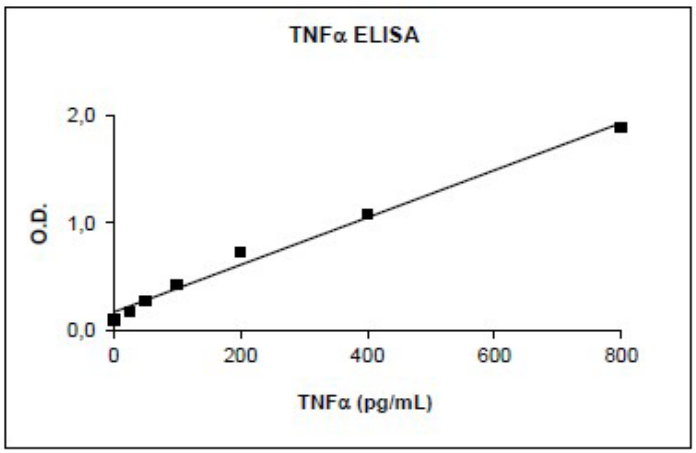
Calculate the average absorbance values for each set of duplicate standards, controls and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding TNFα standard concentration on the horizontal axis.

The amount of TNFα in each sample is determined by extrapolating OD values against TNFα standard concentrations using the standard curve.

Example TNFα Standard Curve

Standard	TNFα Conc (pg/ml)	OD (450nm) mean	CV (%)
1	800	1.883	3.7
2	400	1.076	10.8
3	200	0.724	4.3
4	100	0.43	7.4
5	50	0.277	0.3
6	25	0.18	10.2
zero	0	0.102	4.2



R^2=0.99

Note: Curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill

with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

PERFORMANCE CHARACTERISTICES

1. **SENSITIVITY**: The sensitivity, minimum detectable dose of TNFα using this TNFα ELISA kit was found to be less than **8pg/ml**. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 40 times.
2. **SPECIFICITY:** This assay recognizes both natural and recombinant human TNFα. To assess the specificity of this TNFα ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (IL-1β, IL-6, IL-12, IL-4, IL-2, IFNy, IL-10, IL-8, and IL-13).
3. **PRECISION:**

   Intra-assay

   Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of TNFα: 3 in human pooled serum and 2 in culture media. Data below shows the mean TNFα concentration and the coefficient of variation for each sample. **The overall intra-assay coefficient of variation has been calculated to be 3.2%.**

Session	Sample	Mean TNFα pg/mL	SD	CV%
Session 1	Sample 1	829.33	44.99	5.4
	Sample 2	529.33	10.60	2.0
	Sample 3	201.00	2.00	1.0
	Sample 4	182.33	7.57	4.2
	Sample 5	104.67	1.53	1.5
Session 2	Sample 1	807.00	14.80	1.8
	Sample 2	455.33	12.74	2.8
	Sample 3	171.67	9.07	5.3
	Sample 4	158.00	8.19	5.2
	Sample 5	102.67	4.73	4.6
Session 3	Sample 1	833.00	10.39	1.2
	Sample 2	489.67	12.66	2.6
	Sample 3	172.67	2.52	1.5
	Sample 4	186.67	2.52	1.3
	Sample 5	84.00	6.56	7.8

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two analysts. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of TNFα: 3 in human pooled serum and 2 in culture media. Data below show the mean TNFα concentration and the coefficient of variation for each sample. T**he calculated overall coefficient of variation was 10.9%.**

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean TNFα pg/mL	815	470	167	158	87
SD	31	35	19	25	14
CV%	**3.8**	**7.5**	**11.5**	**15.9**	**16.1**

DILUTION PARALLELISM - Four human pooled serum samples with different levels of TNFα were analyzed at different serial two-fold dilutions (1:2 To 1:8) with four replicates each. Recoveries ranged from 97 to 120% with an overall mean recovery of 107%.

SPIKE RECOVERY - The spike recovery was evaluated by spiking 3 concentrations of TNFα in human serum in 2 separate experiments. Recoveries ranged from 74 to 90% with an overall **mean recovery of 81%.**

STABILITY

Storage Stability

Aliquots of spiked serum and spiked medium were stored at -20°C, +2-8°C, room temperature (RT) and at 37°C and the TNFα level determined after 24h. There was no significant loss of TNFα reactivity during storage at 2-8°C. However there is a little loss when stored at RT and a significant loss of reactivity when stored at 37°C.

Freeze-thaw stability

Aliquots of spiked serum and spiked medium were stored frozen at –20°C and thawed up to 5 times and the TNFα level was determined. There was no significant loss of TNFα after 5 cycles of freezing and thawing.

Expected serum values

A panel of 50 human sera was tested for TNFα. All were below the detection level of 8pg/ml.

Standard Calibration

This immunoassay is calibrated against the International Reference Standard NIBSC 87/650. NIBSC 87/650 is quantitated in International Units (IU). It has been calculated that 1IU corresponding to 75pg TNFα.

REFERENCES

1. Aderka D., et al (1992). J. Exp. Med. 175, 323.
2. Adolf G., and Apfler I. (1991). J. Immunol. Methods 143, 127.
3. Alvaro-Garcia J. M., et al. (1990). J. Clin. Invest. 86, 1790.
4. Beutler B., et al. (1988). Endo. Rev. 9, 57.
5. Cerdan C., et al. (1991). J. Immunol. 146, 560.
6. Chin J. E., et al. (1990). Arthr. Rheum. 33, 162.
7. Cope A. P., et al. (1992). Arthr. Rheu. 35, 1160.
8. Digel W., et al. (1992). J. Clin. Invest 89, 1690.
9. Economou J. S., et al. (1989). Immunol. 67, 514.
10. Engelberts I., et al. (1991). Lancet 338, 515.
11. Gaumond B., et al. (1988). Presented at the 88th Annual Meeting of the American Society for Microbiology.
12. Girardin E., et al. (1988). New Eng. J. Med. 319, 397.
13. Holler E., et al. (1990). Blood 75, 1011.
14. Israel-Biet D., et al. (1991). J. Immunol. 147, 490
15. Janssen O., et al. (1988). J. Immunol. 140, 125.
16. Krishnan V. L., et al. (1990). Clin. Exp. Immunol. 80, 156.
17. Maury C. P. J. (1986). Acta Med. Scan. 220, 387.
18. Maury P. J., et al. (1987). J. Exp. Med. 166, 1137.
19. McLaughlin P. J., et al. (1991). Transplantation 51, 1225.
20. Reddy M. M., et al. (1988). J. Acq. Imm. Def. Synd. 1, 436.
21. Sardas O. S., et al. (1990). Blood 76, 2639.
22. Scuderi P., et al. (1986). Lancet, December 13, 1364.
23. Smith M. R., et al. (1990). J. Immunol. 144, 560.
24. VanZee K. J., et al. (1992). Proc. Natl. Acad. Sci. USA. 89, 4845.
25. Waage A., et al. (1987). Lancet, February 14, 355.

ASSAY SUMMARY

Total procedure length: 3h45min

Add 100µl of Samples, Control and diluted Standards and 50µl diluted Biotinylated Antibody

↓

Incubate 3 hours at room temperature

↓

Wash three times

↓

Add 100µl of diluted Streptavidin-HRP

↓

Incubate 30 min at room temperature

↓

Wash three times

↓

Add 100µl of TMB Substrate
Protect from light. Let the color develop for 12-15 min.

↓

Add 100µl of Stop Reagent

↓

Read Absorbance at 450 nm

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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