GE Healthcare

Amersham Tumor Necrosis Factor Alpha [(h)TNFα] Human, Biotrak ELISA System

96 determinations

Product Booklet

Code: RPN2758

Page Finder

1. Legal

http://www.gelifesciences.com

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage and stability

a. Store all reagents at 2–8°C upon receipt. Do not freeze reagents.

b. Refer to the expiry date stamped on the kit box.

c. Do **NOT** use kit beyond the stated expiry date.

3. Materials

3.1. Materials provided

- Anti-human TNFα precoated stripwell plate, 1, (96 well)
- Lyophilized recombinant human TNFα standard, 2 vials
- Standard diluent, 1 vial (12 ml), with preservative
- Biotinylated antibody reagent, 1 vial (8 ml), with preservative.
- 30x Wash buffer, 1 vial (50 ml)
- Streptavidin-HRP concentrate, 1 vial (0.050 ml), with preservative
- Streptavidin-HRP dilution buffer, 1 vial (14 ml), with preservative
- Premixed TMB substrate solution, 1 vial (12–13 ml)
- Stop solution, 1 vial (13–15 ml), <1% sulfuric acid
- Adhesive plate covers, 4
- Instruction booklet, 1

3.2. Additional materials required

The following materials and equipment are required but not supplied:

- Pipettes with disposable plastic tips to deliver 5–1000 µl.
- Plastic pipettes to deliver 5–15 ml.
- Distilled or deionized water.
- 2 litre measuring cylinder.
- A squirt wash bottle, or an automated immunoplate washer.
- 1.5 ml polypropylene tubes. Do not use polystyrene, polycarbonate or glass tubes.
- A microcentrifuge for preparing streptavidin-HRP solution.
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can

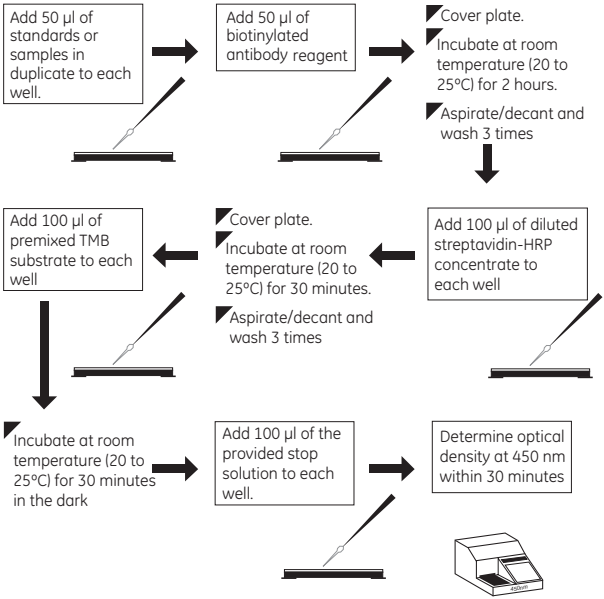
be read only at 450 nm. Refer to the instruction manual supplied with the instrument used.

- Graph paper or a computerized curve-fitting statistical software package.
- Assays may be performed with commercially available microplate washers to aid convenience and assay throughput.

4. Introduction

The Biotrak™ human tumor necrosis factor alpha ELISA from GE Healthcare is an enzyme-linked immunosorbent assay for the quantitative measurement of (h)TNFα in serum, plasma and culture supernatant.

5. Summary of procedure



6. Critical parameters

The following points are critical:

- **All specimens and reagents must be at room temperature (20–25°C) before use in the ELISA.**
- Vigorous washing of plate is essential.
- Appropriate sample diluent must be used when diluting all samples.
- Do not use a 37°C water bath to thaw samples. Thaw samples at room temperature and mix thoroughly before use.
- If using a multichannel pipette, always use a new disposable reagent reservoir for the addition of each reagent.
- Use fresh disposable pipette tips for each transfer to avoid cross contamination.
- Use a new adhesive plate cover for each incubation step in the ELISA.
- Do not mix reagents from different kit lots.
- Avoid handling the tops of the wells both before and after filling.
- Avoid exposure of reagents to excessive heat or light during storage and incubation.
- Individual components of this assay may contain antibiotics and preservatives. Gloves must be worn while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Do not use glass pipettes to measure the TMB substrate solution.
- Care must be taken not to contaminate the TMB substrate solution. If the solution is blue prior to use, **DO NOT USE.**
- A small amount of precipitate may be present in some vials. It will not affect assay performance and should be ignored.
- The total dispensing time for each plate should not exceed 20 minutes.

7. Sample preparation

7.1. Handling and storage

Serum, plasma and culture supernatant may be tested in this ELISA.

- 50 µl of sample or standard per well is required in this assay.
- Samples that are to be assayed within 24 hours should be stored at 2–8°C. When storing samples for longer periods of time, freeze them in aliquots at -70°C.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Avoid repeat freeze thaw cycles when storing samples.
- Bring samples gradually to room temperature before running the assay. **Do not use heated water baths to thaw or warm samples.**
- Mix samples by gently inverting the tubes.

The measurement of cytokines in serum and plasma has been reported to be affected by non-specific matrix effects which may vary between samples from different individuals. Dilution of such samples in the diluent supplied may help to reduce these interference effects.

7.2 Sample dilution

If it is suspected that the (h)TNFα concentration of a sample will exceed the highest point of the standard curve, 1000 pg/ml, prepare one or more five-fold dilutions of the test sample. For example, a five-fold dilution is prepared by adding 50 µl of test sample to 200 µl of the same diluent used to prepare standard dilutions see step 3, page 11. Mix thoroughly between dilutions before assaying. Prepare all sample dilutions using the sample diluent provided.

8. Reagent preparation

8.1. Wash buffer

Label a clean glass or plastic 2 litre container 'Wash buffer.' 30× wash buffer may have a cloudy appearance. Add the entire contents of the 30× wash buffer bottle to the 2 l container and dilute to a final volume of 1.5 litres with distilled or deionized water. Mix thoroughly. **Wash buffer must be at room temperature** prior to use in the assay. If running partial plates, store the reconstituted wash buffer at 2–8°C. **Do not use wash buffer if it becomes visibly contaminated during storage.**

8.2. Standards

1. Two vials of lyophilized standard are provided with this kit. Reconstitute and use one vial per partial plate.

2. Prepare standards shortly before use. Use within one hour of reconstitution. **Do not store reconstituted standards.**

3. Reconstitute standard in distilled or deionized water. The reconstitution volume is stated on the standard vial label. Mix by gently inverting the vial until the contents have completely dissolved (approximately 1 minute) to give a solution of 2.5 ng/ml. When running **culture supernatant** samples, use the culture medium to prepare the standard dilutions as at step 4. below. When running **plasma** or **serum** samples, use the standard diluent provided to prepare the standard dilutions as at step 4. below.

4. Testing of RPMI with different lots and concentrations of fetal bovine serum has shown that this ELISA is not adversly affected by culture media. Therefore, **when running both culture supernatants and serum or plasma on the same plate**, a standard curve reconstituted in water and diluted in standard diluent may be used. If you are using an unusual culture medium you may wish to validate this by running two standard curves in

parallel, with the standard reconstituted in water and then diluted in a) standard diluent and b) culture medium. If the two standard curves are within 10% of the mean for both curves the assay can be run using either curve.

5. Label 6 tubes, one for each standard curve point: 1000, 400, 160, 64, 25.6 and 0 pg/ml. Prepare serial dilutions for the standard curve as follows:
6. Pipette 240 µl of appropriate diluent into each tube.
7. Pipette 160 µl of the reconstituted standard into the first tube, 1000 pg/ml and mix.
8. Pipette 160 µl of this dilution into the next tube, 400 pg/ml and mix.
9. Repeat serial dilutions five more times to complete the standard curve.

8.3. Streptavidin-HRP solution

1. Prepare streptavidin-HRP solution **no more than 15 minutes prior to use.** Do not prepare more than required. See step 5 below when running partial plates.
2. **Do not store prepared streptavidin-HRP solution.**
3. Use a 15 ml plastic tube to prepare the streptavidin-HRP solution. Centrifuge the streptavidin-HRP concentrate to force the entire contents to the bottom of the tube.
4. Add 30 µl of streptavidin-HRP concentrate to 12 ml streptavidin-HRP dilution buffer and mix gently. This is the streptavidin-HRP solution.
5. When running partial plates, use only what is required for the number of strips being run. To run a half plate, add 15 µl streptavidin-HRP concentrate to 6 ml streptavidin-HRP dilution buffer, mix gently.

9. Assay procedure

9.1. Sample and biotinylated antibody reagent incubation

1. Determine the number of strips required. Leave these strips in the plate frame. Place the remaining unused strips back in the foil pouch with the desiccant provided. Store the reserved strips at 2–8°C, making sure the foil pouch is tightly sealed. After running the assay, retain the plate frame for the second partial plate. When running the second partial plate, place the reserved strips securely in the plate frame.
2. Record the locations of the human TNFα standards and test samples. Five standards and one zero must be run in duplicate with each series of unknown samples.
3. Add 50 µl standard or sample to each well in duplicate. Add 50 µl of the biotinylated antibody reagent to all wells being used. Add 50 µl of appropriate sample diluent to any empty wells. **If the TNFα concentration in any test sample exceeds the highest point on the standard curve**, 1000 pg/ml, see section 7.2. Mix well by gently tapping the plate several times.
4. Carefully cover the plate with an adhesive plate cover. Ensure that all edges and strips are sealed tightly by running a thumb over the edges and down each strip. Incubate for 2 hours at room temperature (20–25°C).
5. At the end of the incubation period, carefully remove the adhesive plate cover. Wash the plate **THREE** times with wash buffer, using the procedure outlined in section (9.2).

9.2. Plate washing

Gently squeeze the long sides of the plate frame when washing the plate to ensure that all strips remain securely in the frame.

Manual wash: Decant the contents of the plate into a sink or other receptacle. Using a squirt bottle, **vigorously fill each well completely with wash buffer**, then decant the contents into a sink or other receptacle. Repeat the procedure two more times for a total of **THREE** washes. Blot on to paper towels or other absorbent material.

Automated wash: Aspirate all wells and wash **THREE** times with wash buffer making sure wells are filled with wash buffer. Blot on to paper towels or other absorbent material.

9.3. Streptavidin-HRP solution incubation

1. Add 100 µl of prepared streptavidin-HRP solution, see section (8.3), to each well. If using a multichannel pipette, **use a new reagent reservoir and pipette tips** when adding the prepared streptavidin-HRP solution.
2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate the plate at room temperature (20–25°C) for 30 minutes.
3. At the end of the incubation, carefully remove the adhesive plate cover, decant the plate then wash THREE times using the procedure outlined in section (9.2).

9.4. Substrate incubation and stop step

1. **Use new disposable reagent reservoirs and pipette tips** when adding the TMB substrate solution and stop solution.
2. Dispense from the bottle ONLY the amount required for the number of strips being run, 100 µl per well. **Do not use a glass pipette** to measure the TMB substrate solution. **Do not combine leftover substrate** with that reserved for the second half plate. Care must

be taken to ensure that the remaining TMB substrate solution is not contaminated.

3. Pipette 100 µl of TMB substrate solution into each well.
4. Allow enzymatic color reaction to develop at room temperature for 30 minutes.THE PLATE SHOULD BE DEVELOPED IN THE DARK. **Do not cover the plate with aluminum foil or a plate sealer**. The substrate reaction yields a blue solution that turns yellow when stop solution is added.
5. After 30 minutes, stop the reaction by adding 100 µl of stop solution to each well.

9.5. Reading the absorbance

Measure the absorbance on an ELISA reader set at 450 and 550 nm. Subtract readings of 550 nm from the readings at 450 nm. Reading at dual wavelengths will correct for optical imperfections in the microtitre plate. If a wavelength correction is not available read the plate at 450 nm.

Note: When the 550 nm adjustment is omitted, OD values will be higher. **THE PLATE MUST BE READ WITHIN 30 MINUTES OF STOPPING THE REACTION.**

10. Calculation of results

1. The standard curve is used to determine the amount of human TNFα in an unknown sample. The standard curve is generated by plotting the average absorbance (450–550 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding TNFα concentration on the horizontal (X) axis.
2. Calculate results manually using graph paper or with a curve-fitting statistical software package. If using curve-fitting software, plot a four parameter logistic curve fit. If four parameter is not available, a point-to-point curve fit may be used. The amount of TNFα in each sample is determined by interpolating the TNFα concentration (X axis) from the absorbance value (Y axis). If a dilution was performed on a test sample, multiply the value interpolated from the standard curve by the dilution factor to calculate the amount of TNFα in the sample.
3. Optical density values obtained for duplicates should be within 10% of the mean. **Duplicate values that differ from the mean by greater than 10% should be considered suspect and should be repeated.**

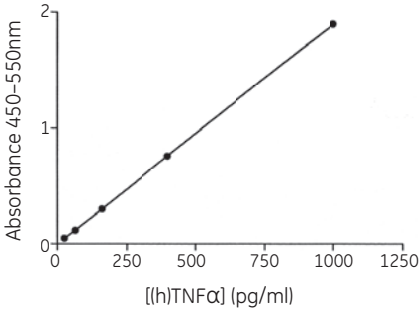


Figure 1. Typical standard curve.

11. Performance characteristics

11.1. Sensitivity

<5 pg/ml

The sensitivity of this assay, or lower limit of detection (LLD)[1], was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

11.2. Assay range

25.6–1000 pg/ml

Standard curve points are 0, 25.6, 64, 160, 400 and 1000 pg/ml.

11.3. Calibration

The standards in this ELISA are calibrated to the WHO reference lot 87/650

1 pg Biotrak standard ≡ 3 pg WHO ≡ ~0.12 WHO unit.

11.4. Reproducibility

Within-assay precision

The within-assay coefficient of variation of the ELISA has been determined to be <10%.

Between-assay precision

The between-assay coefficient of variation of the ELISA has been determined to be <10%.

[1] *Immunoassay:* A Practical Guide, ed. Chan and Perlstein, Academic Press. p.71, 1987.

11.5. Specificity

This ELISA is specific for the measurement of natural and recombinant (h)TNFα. It does not cross-react with the following cytokines: human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IFNα, IFNγ, TNFβ or mouse TNFα.

11.6. Expected values

Normal average levels of (h)TNFα in 9 serum samples is 0 pg/ml.
Normal average levels of (h)TNFα in 9 plasma samples is 0 pg/ml.

12. Related Products

Biotrak range of Easy ELISA systems

Interferon-alpha [(h)IFNα]	RPN5960
Interferon-gamma [(h)IFNγ]	RPN5961
Interleukin 1β [(h)IL-1β]	RPN5971
Interleukin-2 [(h)IL-2]	RPN5965
Interleukin-2 [(m)IL-2]	RPN5966
Interleukin-6 [(h)IL-6]	RPN5968
Interleukin-8/Neutrophil-activating peptide-1-1 [(h)IL-8/NAP-1]	RPN5969
Interleukin-10 [(m)IL-10]	RPN5963
Interleukin-13 [(h)IL-13]	RPN5972
Monocyte chemoattractant protein-1 [(h)MCP-1]	RPN5964
Transforming growth factor beta-1 [(h)TGFβ_1]	RPN5970
Tumor necrosis factor-alpha [(h)TNFα]	RPN5967

Biotrak range of human cytokine and growth factor ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2750
Interleukin-1β [(h)IL-1β]	RPN2751
Interleukin-6 [(h)IL-6]	RPN2754
Interleukin-8 [(h)IL-8]	RPN2764
Interleukin-10 [(h)IL-10]	RPN2755
Interleukin-12 p40/70 [(h)IL-12]	RPN2765
Interferon-gamma [(h)IFNγ]	RPN2757
Vascular Endothelial Growth Factor [(h)VEGF]	RPN2779

Biotrak range of high sensitivity human cytokine and growth factor ELISA systems

Interferon-gamma [(h)IFNγ]	RPN2787
Interleukin-6 [(h)IL-6]	RPN2784
Interleukin-10 [(h)IL-10]	RPN2785
Tumor necrosis factor-alpha [(h)TNFα]	RPN2788

Biotrak range of mouse cytokine and growth factor ELISA systems

Interferon-gamma [(m)IFNγ]	RPN2707
Interleukin-1β [(m)IL-1β]	RPN2720
Interleukin-4 [(m)IL-4]	RPN2712
Interleukin-6 [(m)IL-6]	RPN2708
Interleukin-12 [(m)IL-12]	RPN2702
Tumor necrosis factor-alpha [(m)TNFα]	RPN2718

Biotrak range of rat cytokine and growth factor ELISA systems

Cytokine-induced neutrophil chemoattractant [(r)GRO/C1NC-1]	RPN2730
Interleukin-1β [(r)IL-1β]	RPN2743
Interleukin-6 [(r)IL-6]	RPN2742
Monocyte chemoattractant protein-1 [(r)MCP-1]	RPN2740
Tumor necrosis factor-alpha [(r)TNFα]	RPN2744

Cell Proliferation assay system and reagents

Cell Proliferation Biotrak ELISA	RPN250
Cell proliferation kit for immunocytochemistry	RPN20
Monoclonal anti-bromodeoxyuridine	RPN202
Cell Proliferation Labeling Reagent	RPN201

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