invitron
high sensitivity diagnostics

IV3-104E

English

Tumour Necrosis Factor-α (TNFα) ELISA Kit

For in-vitro diagnostic use only

CE

Invitron Ltd.
Wyastone Business Park, Wyastone Leys
Monmouth NP25 3SR, UK
www.invitron.com

Definitions

 Instructions for use

REF Catalogue number

 Use by

LOT Lot/Batch Code

 Storage temperature limitations

IVD In vitro diagnostic medical device

Contains sufficient for <N> tests

Invitron Tumour Necrosis Factor-α (TNFα) ELISA Kit

Intended Use

The Invitron Tumour Necrosis Factor-alpha (TNF-α) Assay is intended for the quantitative determination of TNF-α in human serum or plasma. This assay is for *in vitro* diagnostic use only.

Summary and Explanation

TNF-α is a 212 amino acid transmembrane protein which is released into the circulation by proteolytic cleavage. It belongs to a group of pro-inflammatory cytokines which exhibit cytotoxic activity. Activated monocytes/macrophages, lymphocytes and natural killer cells as well as mast cells and many malignant cells are capable of producing TNF-α. The protein plays a key role in eliciting inflammatory responses. For example, TNF-α produced by macrophages in synovial fluid induces fibroblast proliferation and also contributes to joint inflammation by stimulating the production of other cytokines such as IL-1 and IL-6. It has been suggested that elevated TNF-α release is associated with the insulin resistance and cardiovascular disease of type 2 diabetics.

Principle

This ELISA is designed to be used for the quantitative determination of TNF-α in serum and plasma. In a first incubation TNF-α is bound to a monoclonal antibody immobilised in the wells of a microtitre plate. After a wash step to remove unreacted substances, a biotinylated monoclonal antibody is added and a second incubation is carried out. After a further wash, a streptavidin-HRP conjugate is added and, after a final wash, the residual enzyme is quantified to provide a measure of bound TNF-α, which is proportional to its concentration in the sample. Quantification is achieved by comparison with TNF-α calibrators.

Materials Provided

- Coated Microtitre Plate
  (8 x 12) stripwells coated with a rabbit antibody.
- Labelled Antibody Concentrate
  (150 µl) Biotinylated mouse anti-TNF-α antibody.
- Conjugate
  (200 µl) peroxidise labelled streptavidin
- Standard
  (3 x 1) vials) lyophilised standard (800 pg).
- Controls
  (3 x 2 vials) lyophilised low and high controls.
- Substrate
  (15 ml) Tetramethylbenzidine (TMB) substrate ready to use.
- Stop Solution
  (15 ml) Dilute sulphuric acid, ready to use.

- Standard Diluent
  (25 ml) ready to use.
- Wash Buffer Concentrate
  (2 x 100 ml) 10x concentration phosphate buffered saline containing detergent and preservative.

Materials Required but not Supplied

- Deionised water
- Calibrated pipettes capable of delivering 10 to 1000 µl
- Plate sealers
- Microtitre plate shaker
- Multi-channel or repeating dispenser
- Vortex mixer
- Microtitre plate reader

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Human materials used in the kit components have tested negative for HIV, hepatitis B and hepatitis C. Nevertheless, for safety reasons, all components should be treated as potentially infective.
- Stop solution is diluted sulphuric acid and therefore must be handled with care. It can cause burns and so gloves, safety glasses and appropriate protective clothing should be worn when handling it. Any spills should be wiped up immediately using large volumes of water.
- Reagents should not be used beyond the expiry date shown on the kit label.

Preparation and Storage of Reagents

- To run the assay more than once, refer to the kit storage conditions indicated on the label. Prepare only the required quantity of reagents for each run. Up to 3 runs may be performed with each kit.
- Reagent vials containing a volume of less than 100 µl should be centrifuged before use.
- Wash buffer concentrate should be diluted 1:10 in deionised water before use. If any crystals have formed during storage, these should be re-dissolved by warming the wash buffer to 37ºC prior to dilution. Wash buffer concentrate is stable when stored at 2-8º up to the expiry date on the label. Diluted wash buffer can be stored in a closed vessel for up to 4 weeks at 2-8ºC.

- Prior to use, the biotinylated antibody is diluted 1:100 in wash buffer. Undiluted antibody is stable when stored at 2-8ºC. **Diluted antibody is not stable and cannot be stored.**

- The streptavidin-peroxidase conjugate must be diluted 1:100 in wash buffer. Undiluted conjugate is stable when stored at 2-8ºC. **Diluted conjugate is not stable and cannot be stored.**

- Lyophilised controls must be reconstituted with 0.5 ml Standard Diluent before use. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion. **Reconstituted controls are not stable and cannot be stored.**

- The lyophilised standard must be reconstituted by addition of 800 µl Standard Diluent. Allow the contents of the vial to dissolve for 10 minutes and mix thoroughly by gentle inversion. The concentration of the reconstituted standard is 1000 pg/ml. To prepare a standard curve, make a series of doubling dilutions in Standard Diluent as follows:

  S1 = 500 µl Stock + 500 µl Standard Diluent
  S2 = 500 µl S1 + 500 µl Standard Diluent
  S3 = 500 µl S2 + 500 µl Standard Diluent
  S4 = 500 µl S3 + 500 µl Standard Diluent
  S5 = 500 µl S4 + 500 µl Standard Diluent
  S6 = 500 µl S5 + 500 µl Standard Diluent
  For zero standard use Standard Diluent
  Reconstituted standards are not stable and cannot be stored

- All other test reagents are ready to use. These reagents are stable until the expiry date given on the kit label when stored at 2-8ºc.

Specimen Collection and Preparation

Plasma and Serum
Serum or plasma samples may be used undiluted. Samples should be frozen as soon as practical after collection and should be stored at -20ºc prior to analysis.

Assay Procedure

1. Bring all components to room temperature before use.

2. Wash the required number of wells 5 times with working strength Wash Buffer (250 µl/well).

3. Add 100 µl of standard, control or sample to the appropriate well. It is recommended that all determinations are carried out in duplicate.

4. Cover with a plate sealer and incubate for 2 hours at room temperature on a plate shaker.

5. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).

6. Add 100µl working strength Biotinylated Antibody solution to each well.

7. Incubate for 1 hour at room temperature on a plate shaker.

8. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).

9. Add 100 µl working strength peroxidise-labelled streptavidin to each well

10. Incubate for 1 hour at room temperature on a plate shaker.

11. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).

12. Add 100 µl TMP substrate to each well.

13. Incubate for 10-20 minutes at room temperature in the dark.

14. Add 50 µl Stop Solution to each well.

15. Measure absorption at 450 nm in an ELISA plate reader.

Results

The results may be calculated automatically using a cubic spline or 4-parameter curve fit. The concentration of the samples can be read directly from this standard curve. Samples with a concentration greater than that of the highest standard should be diluted and re-assayed.

Limitations

- Only if test instructions are rigidly followed will optimum results be achieved.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. If, after checking the above mentioned items, there is no error, contact Invitron directly.

Expected Values

In 40 normal subjects, plasma concentrations were <20 pg/ml.

It is recommended that each laboratory establishes its own reference range.

Performance

Precision

Intra-assay precision of the Invitron TNF-α assay was estimated at a concentration of 155 pg/ml. Twenty estimates produced a CV of 6.3%

Linearity

Dilution of a sample containing TNF-α was linear over the range 10-500 pg/ml.

Sensitivity

Sensitivity of the Invitron TNF-α assay, calculated as 2SD above the zero standard, was 10 pg/ml.

For additional information and product support please contact:

Invitron Limited
Wyastone Business Park, Wyastone Leys, Monmouth NP25 3SR, UK
Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
info@invitron.com
www.invitron.com