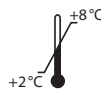


IDK[®] TNF α ELISA

*For the determination of TNF α
in serum, plasma, stool and cell culture supernatant*

Valid from 2022-03-07

REF KR9610



RUO



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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of TNF α in plasma, serum, stool and cell culture supernatant. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Tumor necrosis factor-alpha (TNF α) is a cytokine involved in systemic inflammation. The primary role of TNF α is in the regulation of immune cells. TNF α stimulates the acute phase reaction, induces apoptotic cell death, cellular proliferation and differentiation, inhibits tumor genesis and viral replication. Dysregulation of TNF α production has been implicated in a variety of human diseases.

TNF α is secreted by macrophages, monocytes, neutrophils, T-cells as well as natural killer cells following their stimulation by bacterial lipopolysaccharides. Human TNF α is a non-glycosylated protein with a molecular weight of 17.5 kDa and a length of 157 amino acids. TNF α shows a wide spectrum of biological activities. It causes cytotoxicity and/or cytostasis of many tumor cell lines *in vitro*. Within hours after injection, TNF α leads to destruction of small blood vessels within malignant tumors. TNF α enhances phagocytosis and cytotoxicity in neutrophilic granulocytes and modulates the expression of many other proteins.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9610	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml
KR9610	AB	Antibody concentrate (mouse anti-human-TNF- α , biotinylated)	1 x 150 μ l
KR9610	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 μ l
KR9610	STDKONZ	Standard concentrate, lyophilised (see specification for concentration)	3 x 1 vial
KR9610	CTRL 1	Control, lyophilised (see specification for range)	3 x 1 vial
KR9610	CTRL 2	Control, lyophilised (see specification for range)	3 x 1 vial
KR9610	STDBUF	Standard dilution buffer, ready-to-use	1 x 25 ml

Cat. No.	Label	Kit components	Quantity
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles >0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 3 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- **Preparation of the antibody:** Before use, the **antibody concentrate (AB)** has to be diluted **1:101** in wash buffer (e.g. 100 µl AB+ 10 ml wash buffer). The AB can be used until the expiry date stated on the label when stored at **2–8 °C**. **Antibody (1:101 diluted CONJ) is not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ can be used until the expiry date stated on the label when stored at **2–8 °C**. **Conjugate (1:101 diluted CONJ) is not stable and cannot be stored.**
- The **lyophilised controls (CTRL)** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Reconstitution** details are given in the **specification data sheet**. **Controls (reconstituted CTRL) are not stable and cannot be stored.**
- The **lyophilised standard concentrate (STDKONZ)** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Reconstitution** details, **dilution scheme** and the resulting concentrations needed to prepare a **calibration curve** are given in the **specification data sheet**. **Standards (reconstituted and diluted STDKONZ) are not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Stool samples

For TNFα measurement in stool, please freeze the stool samples at **-20 °C** immediately after collection (not later than two hours after collection). If samples have to be transported, please make sure to keep them frozen.

Serum and plasma samples

Serum/plasma can be used without further dilution. Samples have to be stored at **-20 °C**.

Extraction of the stool samples

Wash buffer (1:10 diluted WASHBUF) is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 0.75 ml sample extraction buffer:

Applied amount of stool:	15 mg
Buffer Volume:	0.75 ml
Dilution Factor:	1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

- The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
- Fill the **empty stool sample tube** with **0.75 ml wash buffer** (1:10 diluted WASHBUF) before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.
- Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
- Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: **1:50**

For analysis, pipet **100 μ l** of the **dilution** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of TNF α .

In a first incubation step, TNF α is bound to monoclonal antibodies, which are immobilised on the surface of the microtiter plate. After a washing step to remove all interfering substances, a biotinylated antibody is added. After another washing step, a horseradish peroxidase-labelled conjugate is added. The amount of the converted substrate by the peroxidase is directly proportional to the amount of bound TNF α and can be determined photometrically at 450 nm. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. TNF α , present in the samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30°C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8°C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Before use , wash the wells 5 times with 250 μl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each 100 μl standards/controls/samples into the respective wells.
3.	Cover the strips and incubate for 2 hours at room temperature (15–30°C) on a horizontal shaker* .
4.	Discard the content of each well and wash 5 times with 250 μl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add 100 μl antibody (diluted AB) into each well.

6.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
7.	Discard the content of each well and wash 5 times with 250 μl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add 100 μl conjugate (diluted CONJ) into each well.
9.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
10.	Discard the content of each well and wash 5 times with 250 μl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add 100 μl substrate (SUB) into each well.
12.	Incubate for 10–20 min** at room temperature (15–30 °C) in the dark .
13.	Add 100 μl stop solution (STOP) into each well and mix well.
14.	Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Stool samples

The obtained results have to be multiplied with the **dilution factor of 50** to get the actual concentrations.

Serum/plasma samples

The actual concentration can be read directly from the results determined (dilution factor 1).

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × sample dilution factor to be used

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 9

The repeatability was assessed with 2 control samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [pg/ml]	CV [%]
1	26.04	3.0
2	90.38	5.9

Reproducibility (Inter-Assay); n = 54

The reproducibility was assessed with 2 control samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [pg/ml]	CV [%]
1	24.43	14.8
2	101.03	9.5

Analytical sensitivity

The following value has been estimated based on the concentrations of the standards without considering possibly used sample dilution factors. For this, the blank value was measured 24 times:

Limit of blank, LoB 4.937 pg/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiry date stated on the kit label.

- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.













14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- IDK® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols:

	Temperature limitation		Catalogue number
	For research use only		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		Irritant