*recovery*ELISA TNFα Neutralization Rate/Adalimumab Kit (RTA)

Catalogue Number: R222 Package Size: 1 kit for 7 samples

1. Description

The r*ecovery*ELISA TNFα Neutralization Rate/Adalimumab Kit (RTA) is an in-vitro diagnostic agent for the simultaneous quantitative in-vitro determination of free TNFα, the TNFα neutralization rate and the available therapeutic antibody Adalimumab in human serum samples. The value "TNFα neutralization rate" indicates the percentage of TNFα which was bound by the therapeutic antibody at the time of blood withdrawal (equivalent to drug activity / Adalimumab activity).
The r*ecovery*ELISA "RTA" consists of a manual, non-automated kit for the determination of 7 samples.
The product is marked with CE. Outside EU territory: Research Use Only.

2. Application

Regular measurement of drug and free TNFα levels or research in therapeutic drug monitoring, pharmacokinetic studies, pharmacodynamic studies, drug development, dose finding trials

3. User community

The test is intended for doctors and specialist personnel in clinical chemical laboratories with experience of conducting immunoassays.

4. Measurement Parameter

(1) Free TNFα level
(2) Available Adalimumab level
(3) Rate of TNFα neutralization in the sample (Adalimumab activity)

5. Species Reactivity

Human

6. Components

Microplate coated with Anti-TNFα-Antibody, Sample dilution buffer, Calibrators for TNFα and for Adalimumab, Control, Anti-TNFα-Antibody-HRP-Conjugate, Washing buffer, TMB Reagent, Stopping solution (1M H_2SO_4)

7. Other materials and equipment required

Multipette and Combitips, 10-100 µL and 100-1000 µL pipettes and tips, vessels for dilution of samples (1 mL volume), timer, measuring cylinder, distilled water, vortexer, microplate shaker, washing device for microplates (where available), microplate reader (450 nm / 620 nm), computer with Microsoft Excel and BioTeZ evaluation software, download from www.biotez.de

8. Principle of the method

The determination of the therapeutic antibody with the *recovery*ELISA is based on the principle that the presence of Adalimumab in patient samples leads to a systematic reduction in the recovery of TNFα [1]. The *recovery*ELISA is an immunological quantitative detection method based on a Sandwich ELISA (Enzyme-Linked Immunosorbent Assay), but a multi-dimensional calibration is carried out obtaining three analysis results within the same assay alongside the interaction curve. The following calibrations are performed:
(1) TNFα levels without and with additional Adalimumab against extinction (optical density)
(2) Adalimumab levels against TNFα recovery.

Robert-Rössle-Str. 10, 13125 Berlin, Germany
Tel.:+49 (0) 30 9489 2130 Fax:+49 (0) 30 949 4509
E-mail: info@biotez.de www.biotez.de

9. Microplate layout

	Strip	1	2	3	4	5	6	7	8	9	10	11	12	
		Field of Calibrators Strip 1-4 TNFα calibrators				**Field of Samples Strip 5-12** **Topping up the samples with TNFα calibrators**								
Row	RTA-TAK calibrators	RTA-TNF-CAL 0	RTA-TNF-CAL 1	RTA-TNF-CAL 2	RTA-TNF-CAL 3	RTA-TNF-CAL 4		RTA-TNF-CAL 2		RTA-TNF-CAL 1		RTA-TNF-CAL 0		**Samples 1:10 dilution**
A	RTA-TAK 0													Sample 1
B														Sample 2
C	RTA-TAK 1													Sample 3
D														Sample 4
E	RTA-TAK 2													RTA-CONTROL
F														Sample 5
G	RTA-TAK 3													Sample 6
H														Sample 7

10. How it works

*recovery*ELISA "RTA" is performed in a 96-well microplate. The wells of the microplate are pre-coated with a capture antibody against human TNFα that binds free TNFα from the patient sample.
There occurs a simultaneous incubation of the TNFα calibrators (with and without the addition of Adalimumab), the diluted samples and the detection conjugate (peroxidase-labeled anti-TNFα signal antibody). The incubation takes place over 16 to 22 h at 2 to 8°C. After washing, the colour substrate TMB (Tetramethylbenzidine) is added to the wells. After incubation the enzymatic colour reaction is stopped using a sulphuric acid solution. A colour change from blue to yellow occurs that is optically detected. Using a wavelength of 450 nm (reference value 620 nm), the optical density (OD) of the reaction product is measured with a suitable microplate reader.

11. Reaction scheme

1. Reaction step	*Incubation of TNFα and Adalimumab calibrators, diluted samples and the detection conjugate at 2-8 °C overnight (min. 16 h, max. 22 h)*
⇩	
Washing step	
⇩	
2. Reaction step	*Enzymatic colour reaction with Tetramethylbenzidin for 30 min at room temperature*
⇩	
Stopping step	*Addition of stop reagent*
⇩	
Measurement at 450 nm	

BioTeZ Berlin-Buch GmbH
Robert-Rössle-Str. 10, 13125 Berlin, Germany
Tel.:+49 (0) 30 9489 2130 Fax:+49 (0) 30 949 4509
E-mail: info@biotez.de www.biotez.de

12. Test evaluation

With the aid of the two calibrations an evaluation procedure can be carried out to determine the concentrations of free TNFα and Adalimumab and the degree of TNFα neutralization in the patient samples using the measured OD values and special BioTeZ software. The evaluation is performed using non-linear regression (Marquardt-Levenberg algorithm) of a logarithmic model from enzyme kinetics and the Langmuir isotherm from surface binding. The results of TNFα are measured in ng/mL and for Adalimumab in µg/mL.

13. Measurement Range

Analyte		Measurement Range
TNFα *	sample without Adalimumab	4 – 400 ng/mL
	sample with Adalimumab	4 – 160 ng/mL
Adalimumab		0.7 – 30 µg/mL

* TNFα-Calibrators in RTA are calibrated to WHO Reference Reagent, NIBSC-Code: 88/786

Please note, that samples are routinely measured with *recovery*ELISA RTA in a 1:10 dilution. Samples whose TNFα or Adalimumab concentrations lie above the measurement ranges should be further diluted and tested again. However, a maximum dilution of 1:30 should not be exceeded to prevent errors occurring in the assay.

14. Disclaimer

*recovery*ELISA "RTA" is not intended at providing treatment recommendations, i.e., whether a patient should receive a drug and if yes, in what kind of dose. The test must be carried out in a laboratory by skilled personnel. The measurement results are handed over to the physician. *recovery*ELISA was developed in collaboration with the pharmaceutical industry only there where it has been explicitly mentioned. Otherwise, the test is independent of all drugs and their conditions of authorization. With approve in-vitro diagnostics, outcomes and the purpose of the tests depend on the respective permission. Where applicable, the scope of services has been reduced, for example of liability considerations. Refer to the respective instruction manual for information.

[1] Clin Chem Lab Med 2012;50(7):1263-1269

BioTeZ Berlin-Buch GmbH
Robert-Rössle-Str. 10, 13125 Berlin, Germany
Tel.:+49 (0) 30 9489 2130 Fax:+49 (0) 30 949 4509
E-mail: info@biotez.de www.biotez.de