English

# i-Tracker Adalimumab

**REF** **CTA 002-100** Σ 100

## INTENDED USE

**i-Tracker Adalimumab** (Theradiag) is an automated assay intended for the quantitative measurement of Adalimumab (anti-TNFα agent) in human serum or plasma samples.

## DIAGNOSTIC VALUE

Anti-TNFα are therapeutic agents widely used to treat patients with various inflammatory diseases. Adalimumab is one of the anti-TNFα recommended for the treatment of the rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, etc. This human monoclonal antibody is able to bind TNFα. It blocks the action of TNFα responsible for the inflammatory state.

However, during the treatment, some patients can develop antibodies against Adalimumab. Consequently, the plasmatic level of anti-TNFα decreases and simultaneously the disease symptoms reappear or increase.

Studies have shown that the trough level of a TNFα therapy (i.e. the circulating drug level just before the next injection) usually correlates clinical efficacy. This trough level is influenced by several factors, among them dosage and frequency of the injections, disease phenotype and activity, pharmacogenetic factors, co-medication and the formation of anti-drug antibodies.

Measurement of anti-TNFα drug level in combination with anti-drug antibodies quantification provides to the treating physician help for therapeutic guidance, hence maximizing treatment efficacy while minimizing to major cost savings.

**i-Tracker Adalimumab** (Theradiag) assay is validated to monitor drug levels of any biological drug which contains the active substance Adalimumab, that is the original drug Humira®, and any biosimilar drug like ABP501 (Amjevita®) and SB5 (Imraldi®).

**i-Tracker Adalimumab** (Theradiag) assay is calibrated with the NIBSC/WHO International Standard (cat:17/236) for Adalimumab.

## SPECIMEN COLLECTION

The specimen types appropriate to **i-Tracker Adalimumab** are human sera and plasma.

Samples which are cloudy should be clarified by low-speed centrifugation.

To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Freshly collected specimens could be tested after storage of at most 8 days kept in refrigerator (+2°C / +8°C) or 3 days at room temperature (+18°C / +25°C) and until 10 hours for onboard specimens.

5 freeze-thaw cycles for specimens do not affect the testing results.

## METHOD PRINCIPLE

**i-Tracker Adalimumab** assay is a two-step immunoassay using microparticles, acridinium-ester labeled chemiluminescent technology with the i-Track[10].

- In the first step, the TNFα coupled magnetic microparticles, and diluted human serum/plasma sample are mixed in an assay cuvette, which allows Adalimumab to bind to the microparticles surface.
- After incubation, unbound reagent and sample matrix are removed by washing, and the microparticles-TNFα-Adalimumab immunocomplex are kept with the help of a magnetic separator.
- Secondly, anti-Adalimumab polyclonal antibodies conjugated to acridinium ester are added.
- After incubation, excess acridinium-ester conjugate is removed by washing and finally the light induced by acridinium-ester is detected by addition of triggers.
- The relative light unit (RLU) intensity is proportional to the amount of Adalimumab.
- According to a certain specific Adalimumab RLU-concentration standard curve, the RLU obtained can be interpreted to Adalimumab concentration in the sample expressed as µg/mL.

For quantitation of Adalimumab, the **i-Tracker Adalimumab** assay uses a predefined lot specific Master Curve that is uploaded into the instrument through the reagent cartridge 2D barcode. The Master Curve is created during manufacturing by using in-house standards. Based on the Master Curve, and results obtained by running two Calibrators, an instrument specific Working Curve is created, which is used to calculate a concentration (µg/mL) from the RLU obtained for each sample.

## KIT CONTENTS

Components are a matched set. **Barcode on the inside box are needed for the assay**.

| Description | Volume |
|---|---|
| **Cartridge reagents** | |
| **Microparticles** : 1 bottle vial of TNFα coupled microparticles in PBS with stabilizers. MP ADA<br>Preservatives: Sodium azide < 0.1% and Proclin 300 < 0.06%<br>***Ready to use*** | 3 mL* |
| **Adalimumab Buffer** : 1 bottle vial of PBS-Tween with stabilizer. BUF ADA<br>Preservatives: Proclin 300 <0.06%<br>***Ready to use*** | 23 mL* |
| **Specimen diluent** : 1 bottle vial of PBS-Tween with stabilizer. DIL ADA<br>Preservative: Proclin 300 <0.06%<br>***Ready to use*** | 23 mL* |
| **Immunoconjugate**: 1 bottle vial of acridinium-ester labeled polyclonal anti-Adalimumab antibodies, containing PBS with stabilizers. IC ADA<br>Preservatives: Sodium azide < 0.1% and Proclin 300 < 0.06%<br>***Ready to use*** | 23 mL* |
| **Control and Calibrators** | |
| **Adalimumab Calibrator A:** a calibrator tube containing Adalimumab (low level) in stabilizers and preservatives. CALA ADA<br>**Target value for the calibrator is indicated on the 2D barcode localized in each kit.**<br>Preservatives: Sodium azide < 0.1% and Proclin 300 < 0.06%<br>***Ready to use*** | 0.5 mL* |
| **Adalimumab Calibrator B:** a calibrator tube containing Adalimumab (high level) in stabilizers and preservatives. CALB ADA<br>**Target value for the calibrator is indicated on the 2D barcode localized in each kit.**<br>Preservatives: Sodium azide < 0.1% and Proclin 300 < 0.06%<br>***Ready to use*** | 0.5 mL* |
| **Adalimumab Control:** a control tube containing Adalimumab in stabilizers and preservatives. CTL1 ADA<br>**Acceptable range for the control is indicated on the 2D barcode localized in each kit.**<br>Preservatives: Sodium azide < 0.1% and Proclin 300 < 0.06%<br>***Ready to use*** | 1mL* |

*When loading a reagent, the i-Track[10] system indicates the remaining usable volume (dead volume is not taken into account).

## MATERIALS REQUIRED BUT NOT PROVIDED

- i-Track[10] (Cat. No. TD 810400)
- Cartridge Checking System (CCS) (Cat. No. TD IS-6010)
- Cuvettes (Cat. No. TD IS-CC100)
- System Liquid (Syst.L) (Cat. No. TD IS-CS100)
- Trigger Set (Cat. No. TD IS-CT100)
- Wash Solution (Wash S) (Cat. No. TD CW100)
- Immunocleaner, (Cat. No. TD IS-IM100)
- D-Sorb Solution (Cat. No. TD IS-DS200)
- Disposable Waste Bags, (Cat No. TD IS-DW225)
- Barcode scanner
- XPrep

## STABILITY AND STORAGE CONDITIONS

- The kit is stable until the expiration date when stored and handled as directed.
- Store the kit in refrigerator (+2°C / +8°C). Open reagents can be kept on board for a maximum of 30 days, or stored at +2°C / +8°C for a maximum of 60 days. When one of the two conditions is reached the reagents are no longer usable.
- The i-Track[10] software monitors the onboard (in-use) expiration of the reagent cartridge. The system will not allow use of a reagent which has expired.

## PRECAUTIONS

1. The product is for in vitro diagnostic use only.
2. This assay is only for use on the i-Track[10].
3. Do not use the reagents beyond their expiration dates. Do not mix reagents from different lots.
4. Instructions must be carefully followed for reagent use and storage. Any modification in procedure may interferes with the results. The control, calibrators and contaminated vials must strictly follow safety guidelines or rules of biological hazards to ensure the users and environmental safety.
5. Reagents contain chemical and biological components. Avoid ingesting or splashing onto skin and mucous membrane. If direct contact with controls happens, rinse immediately the contact area with plenty of water and see a doctor if necessary.
6. Liquid waste and solid waste are temporarily stored on the i-Track[10] in separate containers. Waste management should also be handled in accordance with all federal, state and local environmental regulations when disposing of wastes.

7. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.
8. Once opened, the reagent cartridge must be stored in the instrument's refrigerated reagent racks. Care should be taken to avoid spilling the reagents when the reagent cartridge is reloaded into the instrument.
9. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the i-Track[10] user manual.
10. Reagents can contain Sodium azide < 0.1% and/or Proclin 300 <0.06%. Do not eat and avoid contact with skin and eyes. Azide can form explosive mixtures in copper or lead piping. Rinse thoroughly after flushing.
11.  At this concentration, ProClin 300 is irritating to eyes and skin, and may be detrimental if enough quantity is ingested. It is a skin sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
12. Avoid using reagents if signs of contamination or other visible changes occur.

## ASSAY PROCEDURE

Place the reagent cartridge in the reagent compartment making sure that the barcode is read.

The calibrators and the control must be placed in the sample compartment and make sure that the barcode is read.

Select the appropriate assay protocol on the i-Track[10].

Note that for optimal performance, it is important to perform all routine maintenance procedures, such as routine cleaning, calibration and control procedures which are defined in the i-Track[10] User Manual.

See the i-Track[10] User Manual for preparation, setup, dilutions, adjustment, assay and quality control procedures.

Users should have the periodic calibration procedure for every 21 running days from last calibration, which will be reminded on the software interface. Besides, a calibration procedure should be carried out when a new batch of **i-Tracker Adalimumab** kit is used.

The control procedure could be done before running the specimens every day. Users also can adjust the control procedure period according to their own lab frequency.

### Sample Dilution

The specimens are diluted with Specimen Diluent prior to testing (dilution 1/10) by the i-Track[10] automatically.

## ASSAY CHARACTERISTICS & PERFORMANCES

### *Lower Limit of Quantification (LLOQ) determination*

A population of serum samples from healthy donors or untreated patients were valued by **i-Tracker Adalimumab** assay.

| Lower Limit of Quantification of Adalimumab |
|---|
| 0.5 µg/mL<br>> 95$^{th}$ percentile |

### *Dynamic range & Test Result Interpretation*

With the help of the predicated two-point calibration master curve and a working curve for the instrument specified, the i-Track[10] will automatically calculate the Adalimumab concentration of each specimen and interpret the results into µg/mL.

| Measurement range<br>Adalimumab |
|---|
| 0.5 µg/mL - 24 µg/mL |

Results below the lower limit (< 0.5µg/mL) will be reported as HDM-, while those above the upper limit (> 24 µg/mL) will be reported as HDM+.
Specimen with concentration < 0.5µg/mL is unquantifiable.
Test results only reflect the sample collecting status and should be interpreted/analyzed for diagnosis in conjunction with other laboratory and clinical findings.

### *Interfering Substances studies*

**i-Tracker Adalimumab** (Theradiag) assay was evaluated to assess the impact of potential interfering molecules (bilirubin (0.2 mg/mL), hemoglobin (2 mg/mL), triglycerides (10 mg/mL), rheumatoid factors (1000 IU/mL) and biotin (2000 ng/mL)).
⇨ No interference is detected.

### *Cross Reactivity*

**i-Tracker Adalimumab** (Theradiag) assay was evaluated to assess the impact of potential cross reacting molecules (Infliximab, Certolizumab, Etanercept, Golimumab, Ustekinumab, Vedolizumab and anti-Infliximab antibodies).

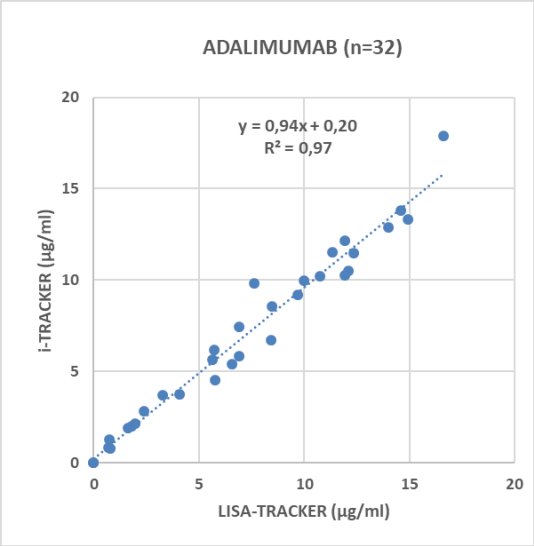
⇨ No cross reactivity is detected.

### *Precision*

| | Intra-run (10 tests in a same assay) | | Inter-run (2 tests in 6 independent assay) | |
|---|---|---|---|---|
| **Parameter** | **Mean (µg/mL)** | **CV (%)** | **Mean (µg/mL)** | **CV (%)** |
| **Adalimumab** | 0.9 | 4.8 | 1.1 | 7.3 |
| | 7.2 | 5.3 | 7.4 | 10.4 |
| | 16.6 | 3.0 | 15.1 | 10.6 |

***Correlation :***

The quantification of Adalimumab performed with **i-Tracker Adalimumab** assay gave similar results than the quantification made with **LISA-TRACKER Adalimumab** assay (Cat.n: LTA 002/LTA 005). A linear regression analysis, generated with 32 Adalimumab samples, shown that both assays are equivalent ($R^2$ = 0.97 ; slope = 0.94).




## LIMITATIONS

The effectiveness of this kit is only confirmed for human sera/plasma, the applicability of the other kinds of samples is not verified.

Reliable and reproducible results will be obtained when the assay procedure is carried out in accordance with the instructions and with adherence to good laboratory practice

Clinical diagnosis should not be made on the findings of a single test result but should integrate all clinical and laboratory findings.

## QUALITY CONTROL

It is recommended to use internally and externally sourced control material. **IMMUNO-TROL i-Tracker Adalimumab** control (Theradiag), (Cat.n: CTA 002-PC) contains **Adalimumab**. This material is to be assayed in the same manner as the unknown sample.

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## SYMBOLS USED

| | | | |
|---|---|---|---|
| CE | EC Declaration of conformity | IVD | *In Vitro* Diagnostic Medical Device |
| REF | Catalogue number | | Manufacturer |
| LOT | Lot Number | | Consult Instruction For Use |
| | Expiry Date | | Temperature limitation |
| Σ | Number of tests | | Biological hazard |
| | Warning | | |

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