Infliximab Total Anti-Drug Antibody ELISA

For the semi-quantitative determination of total anti-drug antibodies against Remicade® in human serum and EDTA plasma.

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Catalog Number: 30-INTHU-E01
Size: 96 Wells
Version: 2025-09-12 – ALPCO 7.0

INTENDED USE

The Infliximab Total Anti-Drug Antibody ELISA is intended for the semi-quantitative determination of human antibodies against TNFα blocker infliximab (e.g. Remicade®) in the presence of infliximab in human serum and EDTA plasma. For Research Use Only in the United States. Health Canada Licensed.

INTRODUCTION

The Infliximab Total Anti-Drug Antibody ELISA measures free and bound antibodies against infliximab (e.g. Remicade®). The assay allows a reliable determination of anti-drug antibodies (ADA) even in the presence of infliximab.

PRINCIPLE OF THE ASSAY

This ELISA serves for the determination of antibodies against the TNF-α-blocker infliximab (e.g., Remicade®). During sample preparation, the anti-drug antibodies (ADA) are separated from the therapeutic antibody to acquire free ADA. By adding the conjugate (peroxidase-labelled therapeutic antibody) and the tracer (biotinylated therapeutic antibody), the unmarked therapeutic antibodies are replaced, and the marked antibodies can form a complex with the ADA. This complex binds via biotin to the streptavidin coated microtiter plate. It is detected via the peroxidase conjugate with the peroxidase converting the substrate TMB to a blue product. The enzymatic reaction is stopped by addition of an acidic solution. The samples convert from blue to yellow. The color change should be measured in a photometer at 450 nm. The interpretation is made using the cut-off control.

MATERIALS SUPPLIED

Component	Quantity	Preparation
Microplate (96 wells)	12 strips of 8 wells	Ready to use
3 Controls (*positive, *negative, cut-off)	4 vials each	Lyophilized
Wash Buffer Concentrate	100 mL	10X
Assay Buffer	2 x 15 mL	Ready to use
Tracer Concentrate, biotinylated	600 µL	12X
Conjugate Concentrate, peroxidase-labelled	600 µL	12X
Antibody Dilution Buffer	10 mL	Ready to use
TMB Substrate	15 mL	Ready to use
Stop Solution	15 mL	Ready to use

*See specification datasheet for range of positive and negative controls.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Disposable precision pipettes for dispensing 10 - 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Standard single-use laboratory glass or plastic vials, cups, etc. for reagent preparation
- Ultrapure* water for reagent preparation *(water type 1;ISO 3696, free of undissolved and colloidal ions and organic molecules (free of particles >0.2um) with an electrical conductivity of 0.055 uS/cm at 25° C (≥18.2MΩcm))*.
- Microplate washer or wash bottle
- Horizontal microplate shaker capable of 550 rpm, orbit 2 mm
- Microplate reader
- Vortex for sample preparation

- Foil to cover the microplate
- Timer

PRECAUTIONS

1. All reagents in this kit are for research use only outside of Canada.
2. The human materials incorporated into this kit have been tested and found to be negative for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). However, for safety reasons, all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
3. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic color 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 ALPCO.
4. The 10x Wash Buffer concentrate contains surfactants which may cause severe eye irritation in case of eye contact.
   Warning: Causes serious eye irritation. If in Eyes: Rinse carefully with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice/attention.
5. The stop solution consists of diluted sulfuric 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 breathe vapor and avoid inhalation.
6. Avoid direct contact with skin.
7. This product is not for internal use.
8. Avoid eating, drinking, or smoking when using this product.

TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is recommended not to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colorless 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.

QUALITY CONTROL

Control samples should be analyzed 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 samples are outside the acceptable limits.

STORAGE CONDITIONS

The unopened kit should be stored at 2-8°C. The unopened kit is stable until the expiration date on the box label.

SAMPLE STORAGE

Undiluted samples can be stored for 2 months at -20˚C or 7 days at 2-8°C or room temperature. Avoid more than 3 freeze-thaw cycles. **Diluted samples** are **not stable** and **cannot be stored**. Sample dilution is carried out simultaneously with the preparation of controls immediately before starting the test.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature (15-30°C) prior to use. To run the assay more than once, ensure that reagents are stored as stated on the label. Prepare only enough reagents necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.

Wash Buffer Concentrate (10X) must be diluted with ultrapure water 1:10 before use (100 mL wash buffer + 900 mL ultrapure water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. The crystals must be redissolved at room temperature or in a water bath at 37°C before dilution of the buffer solution. The undiluted wash buffer is stable at 2-8°C until the expiry date stated on the label. 1X working wash buffer (1:10 diluted wash buffer) can be stored in a closed container at 2-8 °C for 1 month.

Tracer and Conjugate Concentrate (12X) must be diluted together with antibody dilution buffer 1:12 immediately before use (3,000 µL antibody dilution buffer + 300 µL tracer + 300 µL conjugate) and mixed well. Undiluted tracer and conjugate are stable at 2-8°C until expiry date stated on the label. 1x working tracer and conjugate are not stable and cannot be stored.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8 °C.

Sample and Control Preparation – PREPARE IMMEDIATELY PRIOR TO ASSAY
NOTE: Prepared samples must not be stored and must be analyzed immediately in the assay!

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

1. Dilute samples 1:10 in assay buffer by pipetting 25 µL sample into a reaction tube and adding 225 µL assay buffer and vortex. ATTENTION: **Addition of the assay buffer to the samples should be performed as soon as possible for all samples, since this step cleaves the ADAs from the therapeutic antibodies.**
2. Reconstitute the controls with 500 µL assay buffer and vortex. ATTENTION: Carry out this step simultaneously with sample dilution to ensure equal treatment of controls and samples.
3. Incubate controls and diluted samples in reaction tubes for 20 min at room temperature (15-30°C).
   CAUTION: Incubation time begins upon addition of assay buffer.
4. At the end of the incubation period, transfer 250 µL of each control into a reaction vessel.
5. Add 60 µL tracer/conjugate/antibody dilution buffer solution (see reagent preparation) to 250 µL control/diluted sample. Vortex and incubate for 1 hour at room temperature (15-30°C).

The samples are now ready for the assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (15-30°C) and mix well. Mark the positions of controls/samples on a protocol sheet. Take as many microtiter strips as needed from the kit. Store unused strips together with desiccant closed in the aluminum packaging at 2-8°C. Strips are stable until the expiry date stated on the label.
All controls and samples should be run in duplicate.

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 ALPCO.

1. Before use, wash the wells of the coated microtiter plate 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2. Pipet 100 µL of preincubated controls and samples into the wells of the microtiter plate.
3. Cover plate and incubate for **1.5 hours with shaking** on a horizontal shaker* at room temperature (15-30°C).
4. Discard the contents of the wells and wash each well 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5. Add 100 µL of TMB substrate solution into each well.
6. Incubate for 10–20 minutes at room temperature (15-30°C) until a sufficiently large difference in color occurs.**
7. Add 100 µL stop solution into each well and mix quickly by using the **shake function** of the microplate reader.
8. Determine the absorption **immediately** with the microplate reader at 450 nm against 620 nm (or 690 nm) as a reference wavelength.

*It is recommended to set plate shaker to 550 rpm with an orbit of 2mm.

**The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

RESULTS

Cut-off = 10 AU/mL = OD cut-off control

Samples which have a higher average optical density (OD) than the cut-off control are positive.

A linear regression with a linear ordinate and abscissa for optical density and concentration is recommended to calculate the results. The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the program used, a comparison of the paired values should be done manually.

Sample calculation for a positive sample

Average OD of sample = 0.735
Average OD of cut-off control = 0.085 = 10 AU/mL
Concentration of sample = (0.735 × 10 AU/mL)/0.085 = 86.47 AU/mL

LIMITATIONS

Measurement Range
The lower limit of the measurement range is the LoQ.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

Biotin Interference
Samples containing a biotin concentration of <100 ng/mL show a change of the results ≤ 25%. Higher concentrations of biotin can lead to falsely low results. Subjects taking >5 mg biotin per day should wait at least 24 hours after taking biotin to have samples collected.

Infliximab Interference
Samples containing an Infliximab concentration of ≤ 10 µg/ml show a change of results of ≤ 25%, based on samples and the antibody 19/234 from the WHO International Standard "1st Infliximab Antibody Reference Panel" (NIBSC 22/272). Higher concentrations of Infliximab can lead to falsely low results.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Limit of blank, LoB	3.8 AU/mL
Limit of detection, LoD	5.4 AU/mL
Lower limit of quantitation, LoQ	5.9 AU/mL

The specified accuracy goal for the LoQ was 10% CV.

Precision and Reproducibility

Repeatability (Intra-assay); n = 41

The repeatability was assessed with 2 serum samples under constant parameters (same operator, measurement system, day, and kit lot).

Sample	Mean Value (AU/mL)	CV (%)
1	54.1	3.2
2	219.5	4.7

Reproducibility (Inter-assay)

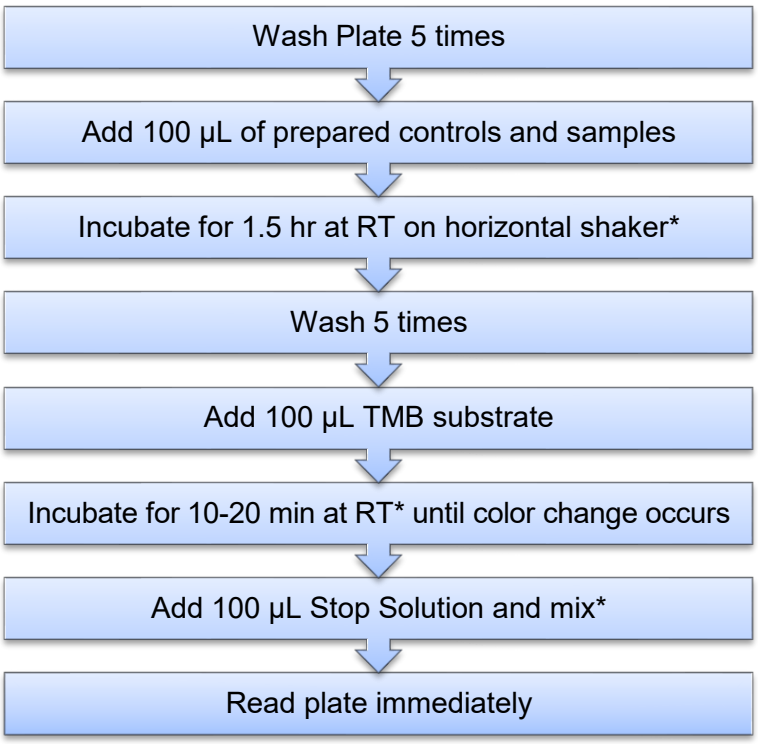
The reproducibility was assessed with 3 serum samples under varying parameters (different operators, measurement system, days, and kit lots).

Sample	Mean Value (AU/mL)	CV (%)
1 (n = 32)	56.0	8.8
2 (n = 32)	62.2	9.2
3 (n = 20)	9.5	6.1

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- 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 manufacturer, may influence the results of the test. ALPCO cannot 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.

SHORT ASSAY PROTOCOL



Total Incubation Time = 1 hour 50 minutes
*Plate Shaker Settings 550 rpm, orbit 2 mm

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running controls and up to 45 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ctrl 1	Ctrl 1	6	6	14	14	22	22	30	30	38	38
B	Ctrl 2	Ctrl 2	7	7	15	15	23	23	31	31	39	39
C	Ctrl 3	Ctrl 3	8	8	16	16	24	24	32	32	40	40
D	1	1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	45	45

Ctrl = Control
Numbered wells = Samples