Adalimumab Total Anti-Drug Antibody ELISA

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

For Research Use Only in the United States. Not For Use In Diagnostic Procedures.

Health Canada Licensed.

Catalog Number: 30-ADTHU-E01

Size: 96 Wells

Version: 2019-02-14 – ALPCO 2.0

INTENDED USE

The Adalimumab Total Anti-Drug Antibody ELISA is intended for the semi-quantitative determination of total anti-drug antibodies against adalimumab (Humira®) in human serum and EDTA plasma. For research use only in the United States. Health Canada Licensed.

PRINCIPLE OF THE ASSAY

This ELISA is designed for the semi-quantitative determination of antibodies against TNF-alpha blocker adalimumab (Humira®). During sample preparation, the anti-drug antibodies (ADA) are separated from the therapeutic antibody to acquire free ADA. By adding the peroxidase 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 adding 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 x 8 strips	Ready to use
3 Controls (positive, negative, cut-off)	4 vials each	Lyophilized
Wash Buffer Concentrate	1 x 100 mL	10X
Assay Buffer	2 x 15 mL	Ready to use
Tracer Concentrate	1 x 600 µL	12X
Conjugate Concentrate	1 x 600 µL	12X
Antibody Dilution Buffer	1 x 10 mL	Ready to use
TMB Substrate	1 x 15 mL	Ready to use
Stop Solution	1 x 15 mL	Ready to use

MATERIALS REQUIRED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 550 rpm, orbit 2 mm
- Microplate reader
- Centrifuge (1,000 xg to 3,000 xg)
- Vortex for sample preparation
- Laboratory Balance
- Foil to cover the microplate
- Timer

PRECAUTIONS

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, 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.
2. All materials derived from animal sources are bovine spongiform encephalopathy (BSE) negative. However, all materials should be treated as potentially infectious.
3. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
4. 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.
5. Avoid direct contact with skin.
6. This product is not for internal use.
7. Avoid eating, drinking, or smoking when using this product.
8. Do not pipette any reagents by mouth.
9. Reagents from this kit are lot-specific and must not be substituted.
10. Do not use reagents beyond the expiration date.
11. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

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

SAMPLE HANDLING

Serum and EDTA Plasma samples are appropriate for use in this assay.
The sample stability is as follows: Undiluted samples can be stored 1 month at - 20 °C or 7 days at 2-8°C. Diluted samples are not stable and cannot be stored.

SAMPLE AND CONTROL PREPARATION

1. Dilute samples 1:10 in assay buffer by pipetting 25 µL of sample into a reaction tube and adding 225 µL assay buffer. Mix well. Addition of the assay buffer to the samples should be performed without pause since this step dissociates the antibody–therapeutic antibody complexes.
   Reconstitute the controls with 250 µL assay buffer and vortex. Carry out this step simultaneously with sample dilution in order to ensure equal treatment of controls and samples.
2. Incubate controls and diluted samples in reaction tubes for 20 min shaking* on a horizontal shaker at room temperature (15–30 °C).
   CAUTION: Incubation time begins upon addition of assay buffer.
3. Add 60 µL tracer/conjugate/antibody dilution buffer solution (see Reagent Preparation section) to 250 µL of control/diluted sample. Vortex and incubate for 1 hour shaking* on a horizontal shaker at room temperature (15–30 °C).

*It is recommended to shake the controls and reaction tubes at 550 rpm with an orbit of 2 mm.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

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

Tracer and Conjugate (12X) must be diluted together with antibody dilution buffer 1:12 immediately before use (3000 µL antibody dilution buffer + 300 µL tracer + 300 µL conjugate), mix well. Undiluted tracer and conjugate are stable at 2–8 °C until expiry date stated on the label. Diluted 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) when stored at 2–8 °C.

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.

ASSAY PROCEDURE

Take as many microtiter strips as needed from the kit. Store unused strips in the aluminum pouch at 2–8° C. Strips are stable until expiry date stated on the label.

Bring all reagents, selected microtiter strips, and samples to room temperature (15–30°C) and mix well. Mark the positions of controls and samples on a plate map. 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 coated microtiter plate 5 times with 250 µL wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the microtiter plate on absorbent paper.
2. Add 100 µL of preincubated controls/samples into the respective wells of the microtiter plate.
3. Cover the plate and incubate for 1.5 hours shaking on a horizontal mixer* at room temperature (15–30 °C).
4. Discard the contents of the plate and wash each well 5 times with 250 µL wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the microtiter plate on absorbent paper.
5. Add 100 µL of TMB substrate solution into each well.
6. Incubate for 10–20 minutes shaking* at room temperature (15–30 °C) in the dark**.

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 reference wavelength.

*It is recommended to shake the microtiter plate on a horizontal mixer set to 550 rpm, with an orbit of 2 mm.

**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.

It is recommended to use a linear regression with a linear ordinate and abscissa to calculate the results.

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

The lower limit of the measurement range can be calculated as:
Limit of Blank (LoB) × sample dilution factor to be used

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

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The following value has been determined without considering possibly used sample dilution factors.

The LoB (limit of blank): 2.6 AU/mL.

Accuracy-Precision

Repeatability (Intra-assay); n = 23
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	59.9	3.5
2	290.9	5.9

Reproducibility (Inter-assay); n = 14
The reproducibility was assessed with 2 serum samples under varying parameters (different operators, measurement systems, days, and kit lots).

Sample	Mean value AU/mL	CV (%)
1	17.4	7.1
2	48.1	5.2
3	213.5	5.1

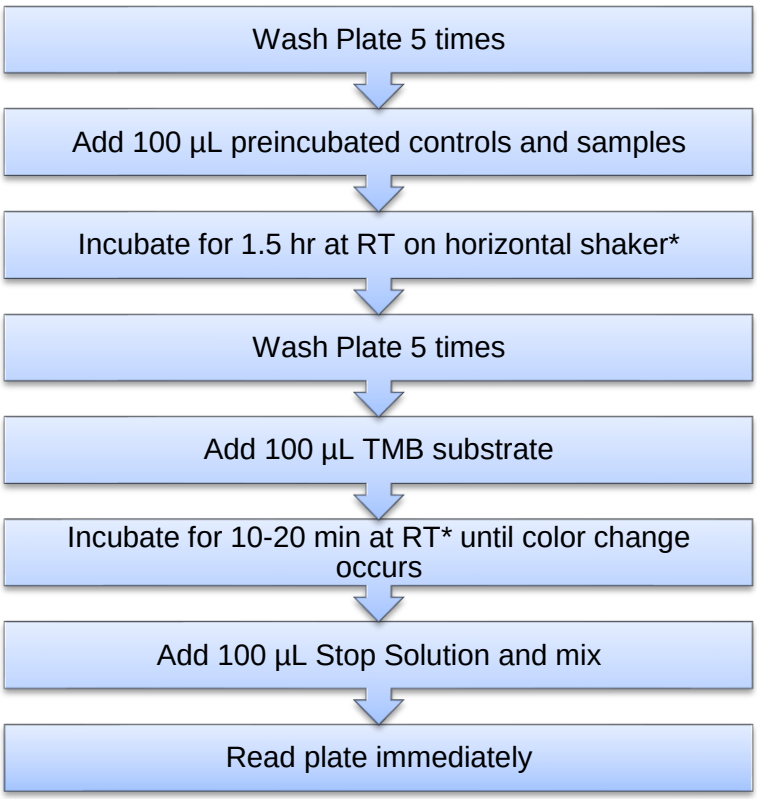
TECHNICAL HINTS

- 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.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for medical 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 producer, may influence the results of the test.

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