Etanercept Free Anti-Drug Antibody ELISA

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

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

Catalog Number: 30-ETFHU-E01

Size: 96 Wells

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INTENDED USE

The ALPCO Etanercept Free Anti-Drug Antibody ELISA is intended for the semi-quantitative determination of free anti-drug antibodies against etanercept (ENBREL®) in human serum and EDTA plasma. For Research Use Only. Not for diagnostic use.

PRINCIPLE OF THE ASSAY

This ELISA is designed for the determination of free antibodies against etanercept (e.g. ENBREL®). In a first incubation step, the free anti-etanercept antibodies from the sample are bound to the etanercept coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, peroxidase-labeled antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stop solution is then added. The color converts to yellow. The absorbance of the color compound is determined photometrically at 450 nm. The intensity of the color is directly proportional to the amount of bound anti-etanercept antibodies (e.g. ENBREL®) from the sample. The results are evaluated using a cut-off control.

MATERIALS SUPPLIED

Component	Quantity	Preparation
Microplate (96 wells)	12 x 8 strips	Ready to use
2 Controls (positive, negative)	4 vials each	Lyophilized
Wash Buffer Concentrate	100 mL	10X
Sample Buffer	30 mL	Ready to use
Conjugate Concentrate	200 µL	101X
TMB Substrate	15 mL	Ready to use
Stop Solution	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 700-900 rpm
- 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.

Storage: Freshly collected EDTA plasma or serum can be stored for one day at room temperature (15–30°C). For longer storage keep the samples frozen at -20°C.

SAMPLE PREPARATION

EDTA plasma or serum samples must be diluted **1:10** before performing the assay, e. g.

25 µL sample **+ 225 µL** Sample Buffer, mix well.

For analysis, pipet **100 µL** of each diluted sample per well. It is recommended to run the samples in duplicate which requires **2 x 100 µL** per sample.

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 stock solutions. The crystals must be dissolved at room temperature or in a water bath at 37°C before dilution of the buffer solutions. The undiluted wash buffer is stable at 2–8°C until the expiry date stated on the label. Wash buffer (1:10 diluted wash buffer) can be stored in a closed flask at 2–8°C for one month.

Controls are provided as lyophilized components. They must be reconstituted with 500 µL of distilled water. Allow the vial contents to dissolve for 10 minutes at room temperature, and mix thoroughly by gentle inversion to ensure complete reconstitution. The undiluted controls are stable at 2–8°C until the expiry date stated on the label. Reconstituted controls cannot be stored.

Conjugate Concentrate (101X) must be diluted with wash buffer 1:101 before use (100 µL conjugate concentrate + 10 mL wash buffer) and mixed well. The conjugate concentrate is stable at 2–8°C until the expiry date stated on the label. Diluted conjugate (1:101 diluted) is 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.

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

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All controls and samples should be run in duplicate.

Take as many microtiter strips as needed from kit. Store unused strips covered 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 ALPCO.

1. Wash the microtiter strips 5x with 250 µL wash buffer before use. After the final washing step, the inverted microtiter strips should be tapped on absorbent paper.
2. Add 100 µL of the controls and diluted samples in the respective wells of the microtiter plate.
3. Seal the strips with foil and incubate for 2 hours at room temperature (15–30°C), on a horizontal mixer set to 550 rpm, amplitude 2 mm.
4. Aspirate the content of the plate and wash each well 5x with 250 µL wash buffer. After the final washing step, the inverted microtiter should be firmly tapped on absorbent paper.
5. Add 100 µL conjugate into each well.
6. Seal the strips with foil and incubate for 1 hour shaking on a horizontal mixer set to 550 rpm, amplitude 2 mm at room temperature (15–30°C).
7. Aspirate the contents of the plate and wash each well 5x with 250 µL wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8. Add 100 µL of TMB substrate solution into each well.
9. Incubate for 5-15 minutes at room temperature in the dark. *
10. Add 100 µL stop solution into each well and mix well.
11. Determine absorption immediately with an ELISA reader at 450 nm. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e.g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

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

RESULTS

The results are evaluated by a **cut-off value** which is estimated by multiplying the optical density (OD) of the **negative control** by **4**.

Samples with a mean OD above the cut-off value are positive.

Samples with a mean OD below the cut-off value are negative.

Example:

OD (negative control)	= 0.011
Cut-off value	= 0.011 x 4 = 0.044
OD (Sample) > 0.044	= positive
OD (Sample) ≤ 0.044	= negative

LIMITATIONS

Samples which cannot be clearly interpreted (e.g. because of high coefficients of variation of replicates) should be measured again.

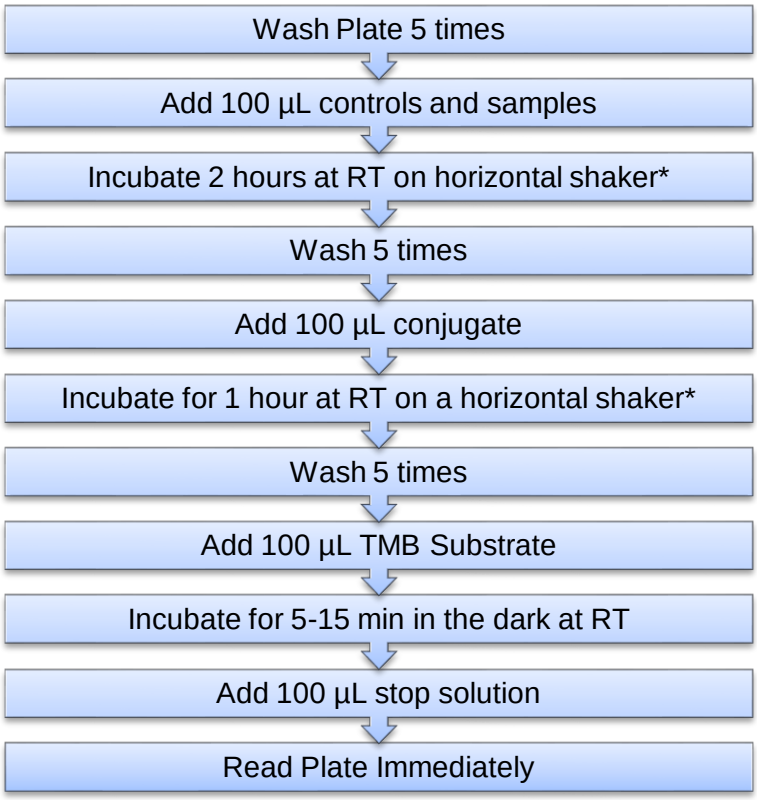
TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended 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 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 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 = 3 hours and 15 minutes
*Plate Shaker Settings: 550 rpm, amplitude 2 mm

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running controls and up to 46 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ctrl 1	Ctrl 1	7	7	15	15	23	23	31	31	39	39
B	Ctrl 2	Ctrl 2	8	8	16	16	24	24	32	32	40	40
C	1	1	9	9	17	17	25	25	33	33	41	41
D	2	2	10	10	18	18	26	26	34	34	42	42
E	3	3	11	11	19	19	27	27	35	35	43	43
F	4	4	12	12	20	20	28	28	36	36	44	44
G	5	5	13	13	21	21	29	29	37	37	45	45
H	6	6	14	14	22	22	30	30	38	38	46	46

Ctrl = Control
Numbered wells = Samples