MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Adalimumab trough levels ELISA Kit

Product Number: ELK010

Shipping and Storage

1. Store the kit at 2~8°C. Do not freeze.
2. Unused microplate strips should be sealed and stored dry.

Component

Component	96T
Pre-coated microplate (breakable strips) with Adalimumab conjugate antigen	1 plate(12 holes×8 strips)
Adalimumab Standard solutions	6 bottles (1ml/bottle)
Anti-Adalimumab Antibody Enzyme Conjugate	6mL
Chromogen Solution A	6mL
Chromogen Solution B	6mL
Stop Solution	6mL
Sample Diluent	(10×, 6mL)
Concentrated Wash Buffer	(20×, 6mL)

Description

This kit employs a competitive ELISA method. The microplate is pre-coated with Adalimumab conjugate antigen. Adalimumab standards or samples are added. Free Adalimumab and the Adalimumab conjugate antigen pre-coated on the microplate wells compete for the anti-Adalimumab antibody enzyme conjugate. After adding TMB substrate for color development and then stop solution, the color changes from blue to yellow. The absorbance is measured at 450 nm using a microplate reader. The absorbance value is inversely proportional to the Adalimumab concentration in the sample. The Adalimumab concentration in the sample is calculated based on the standard curve.

Application

This kit is designed for the quantitative detection of adalimumab residues in meat tissue, serum, and urine samples.

Materials required but not supplied

1. Equipment: Microplate reader with 450nm filter, Homogenizer/Blender, Graduated cylinder, Shaker/Vortex mixer, Funnel, Whatman No. 1 or equivalent filter paper, Micropipettes.
2. Reagents: Deionized water or distilled water, Methanol.

Note

1. Please read the instructions carefully before using the kit.
2. Do not use expired kits.
3. Before use, bring all reagents to room temperature (25±2°C). It is recommended to equilibrate for at least 2 hours.
4. The standards contain Adalimumab. Handle with special care. Wear gloves during operation.
5. The stop solution contains sulfuric acid. Prevent skin burns and corrosion of clothing.
6. Do not reuse pipette tips for different standards or samples, as this may affect the test results.
7. Do not mix reagents from different kit lots; do not reuse pipette tips for different standards or samples, as this may affect the test results.
8. The sample diluent provided in this kit must be used for diluting samples; otherwise, results may be affected.

9. Avoid generating bubbles when mixing reagents.

Reagent Preparation

1. Adalimumab Standard Solutions: 0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb.
2. Concentrated Wash Buffer: Dilute 1:20 (1+19) with distilled water before use.
3. Sample Diluent: Dilute 1:10 (1+9) with distilled water before use.
4. Stop Solution: Ready to use.

Sample Processing Procedure

Strictly follow the instructions during sample extraction. Accurate dilution during extraction is crucial; otherwise, results may be inaccurate. Store samples in a cool, dark place and under refrigeration.

1. Take 10g of homogenized sample, add 20ml of 70% Methanol solution.
2. Vigorously shake for 3 minutes.
3. Filter using Whatman No. 1 filter paper.
4. Take 25µL of the processed sample and add 25µL of Sample Diluent to the reaction well (sample dilution factor is 2).

Protocol

1. Notes before Assay

1.1. Before starting the experiment, equilibrate all reagents outside the box to room temperature (25±2°C) for approximately 2 hours. Remove the required microplate strips only after equilibration. Immediately reseal and store any unused strips dry at 2~8°C.

Note: Ensure thorough equilibration, otherwise detection precision and accuracy will be affected.

1.2. Return reagents to 2~8°C immediately after use.

1.3. Do not modify the assay procedure.

1.4. Use precise micropipettes.

1.5. Once the operation starts, do not interrupt any step.

1.6. The reproducibility of ELISA results depends heavily on the operational procedure; please follow the requirements strictly.

1.7. To avoid cross-contamination, use separate pipette tips for each standard and sample.

1.8. When adding solutions, do not let the pipette tip touch the liquid or inner surface of the well.

2. Assay Procedure

2.1. Pre-label the positions for B0, standards, and samples. Duplicate wells are recommended.

2.2. Remove the required number of microplate strips (removable). Reseal the unused strips and immediately return them to 2~8°C storage.

2.3. Dilute the Sample Diluent (10x) and Concentrated Wash Buffer (20x) to working solutions using distilled or deionized water.

2.4. Add 50µL of the 0.0 ppb standard solution to the B0 well(s).

2.5. Add 50µL of each standard solution to the respective standard wells.

2.6. Add 50µL of each sample solution to the respective sample wells.

2.7. Add 50µL of the Anti-Adalimumab Antibody Enzyme Conjugate to ALL wells.

2.8. Gently shake the reaction plate for a few seconds.

3. Incubate at 37°C for 30 min (Gently tap the plate intermittently during incubation to reduce well-to-well variation).

Discard the liquid from the wells. Wash the microplate 5 times with Wash Buffer. After the last wash, blot the plate firmly onto absorbent paper to remove residual liquid.

4. Reaction

4.1. Immediately after the washing step, add 50µL of Chromogen Solution A to each well, followed by 50µL of Chromogen

Solution B, using a micropipette. Gently shake the plate to mix thoroughly.

4.2. Incubate at 37°C for 10 min.

4.3. Add 50µL of Stop Solution to each well and mix.

4.4. Measure the absorbance at 450 nm. Read the results within 5 minutes.

Result judgment

1. Quantitative Analysis

1.1. Calculate the average absorbance (B) for each standard and sample. Divide this value (B) by the average absorbance of the zero standard (B0) and multiply by 100% to obtain the Percent Absorbance (%B/B0).

B = Average absorbance of standard or sample solution

B0 = Average absorbance of the 0 ppb standard solution

1.2. Plot the standard curve using the log10 of the Adalimumab concentration on the X-axis and the % absorbance on the Y-axis. For each sample, find the corresponding concentration on the curve using its % absorbance. The concentration read from the curve is the log value; take the antilog to obtain the concentration C (ppb) in the assay solution.

1.3. As the sample was pre-diluted, the concentration obtained from the standard curve must be multiplied by the corresponding dilution factor.

2. Semi-Quantitative Determination

2.1. Visual Semi-Quantitative Determination: Select an appropriate standard to run concurrently with the samples. Compare the color intensity of the sample with that of the standard to determine if the sample concentration is less than or greater than the standard value.

2.2. Instrumental Semi-Quantitative Determination: Select an appropriate standard to run concurrently with the samples. Compare the absorbance value of the sample with that of the standard to determine if the sample concentration is less than or greater than the standard value.

Specificity

Substance	Cross-Reactivity
Adalimumab	100%

Kit Parameters

1. The detection limit of this kit is 0.05ppb.
2. The optimal absorbance value for B0 should be greater than 1.0.
3. The intra-assay Coefficient of Variation (CV) is less than 8%, and the inter-assay CV is less than 15%.
4. The recovery rate using the tissue sample extraction method provided in this manual is greater than 80%.
5. The standard curve range provided by the kit is 0.1ppb ~ 8.1ppb.

Analysis Limitations

Samples testing positive with this kit should be confirmed by an alternative method, such as HPLC or GC/MS.