ab287807 – Human Alanine Aminotransferase (ALT) ELISA Kit

For quantitative measurement of human ALT in serum, plasma and cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287807

Storage and Stability

An unopened kit can be stored at 4°C for 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Micro Elisa Plate	8 wells × 12 strips	4°C
Lyophilized Standard	2 vials	4°C
Sample / Standard Dilution Buffer	20 ml	4°C
Biotin-Detection Antibody (Concentrated)	120 µl	4°C
Antibody Dilution Buffer	10 ml	4°C
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µl	4°C
SABC Dilution Buffer	10 ml	4°C
TMB Substrate (Avoid light)	10 ml	4°C
Stop Solution	10 ml	4°C
Wash Buffer (25X)	30 ml	4°C
Plate Sealers	5	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Δ Note: *Prepare reagents within 30 minutes before the experiment.*

Biotin-detection antibody working solution: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with an additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer to make 750 ml of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm it with a 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation

1. Reconstitute the lyophilized GPT standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 200 mIU/ml standard stock solution. Use within 2 hours after reconstituting.
2. Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
3. Prepare 0.6 ml of 100 mIU/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay. Suggested standard points are: 200, 100, 50, 25, 12.5, 6.25, 3.125, and 0 mIU/ml.

Sample Preparation

Samples should be assayed within 5 days when stored at 4°C, otherwise aliquot and store at -20°C (≤1 month) or - 80°C (≤ 2 months). Avoid repeated freeze-thaw cycles.
End users should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

Serum: Coagulate the serum for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.

Tissue homogenates: Rinse the tissues with ice-cold PBS (0.01 M, pH 7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed, minced to small pieces, and then homogenized in PBS with a glass homogenizer on ice. The volume of PBS required depends on the weight of the tissue; 9 ml PBS would be appropriate for 1 g of tissue. It is recommended to add some protease inhibitor into the PBS. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurities and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run with each assay.

1. Prepare all reagents, samples, and standards as previously instructed.
2. Wash plate 2 times with 1X Wash Solution before adding standard, sample and control wells.
3. Add 100 µl of each standards or samples into appropriate wells. Cover well and incubate for 1.5 hours at 37°C.
4. Remove the cover and discard the plate content without washing or letting the wells completely dry.
5. Add 0.1 ml of Biotin-detection antibody work solution into the above wells. Seal the plate and incubate at 37°C for 60 min.

6. Discard the solution and wash 3 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or auto-washer. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
7. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
8. Discard the solution and wash 5 times with 1X Wash Solution, as in step 6.
9. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37 °C in dark for 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
10. Add 50 µl of Stop Solution to each well. Read result at 450 nm within 20 minutes.

Calculation

- Determine the average of the duplicate readings for each standard and samples.
- Calculate the relative O.D.450 of each reading by subtracting the O.D.450 of the zero well, using the equation below.
- The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X).
- If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.
- The Human GPT concentration of the samples can then be interpolated from the standard curve.

The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well)

Technical Support

For all technical or commercial enquiries please go to:
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