Human Cholesteryl Ester Transfer Protein (CETP) ELISA Kit

Cat.No: MBS017656

Store All Reagents At 2°C-8°C !

Package Size: 96 T/Kit

Valid Period: Six Months (2°C-8°C)

For Samples: Serum, Plasma, Tissue Homogenate, Feces, Urine and Body Fluids

IN VITRO RESEARCH USE ONLY! NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

1. INTRODUCTION AND INTENDED USE

ELISA is a simple and highly sensitive method of analysis that allows for simultaneous and rapid quantification of a large number of samples. The assay is based on the specific recognition of the target compound (analyte/antigen) by antibodies which bind to the compound. The antigen-antibody complex is detected and measured with the aid of an enzyme-labeled antibody or antigen. Upon addition of a non-colored reagent, the enzyme produces a color reaction where the color intensity is directly or inversely proportional to the concentration of the analyte in the sample. This quantitative Sandwich ELISA kit is in tended to determinate CETP concentrations in Human serum, plasma, tissue homogenates, feces, urine and body fluids, and it is only for research, not for drug, household, therapeutic or diagnostic applications!

2. PERFORMANCE

Sensitivity: The sensitivity of this kit is 0.1 ug/ml.

Detection Range: The detection range of this kit is 0.25 ug/ml – 8 ug/ml.

Specificity: No significant cross-reactivity or interference between Human CETP and analogues was observed.

Reproducibility: Both Intra-assay CV (%) and Inter-assay CV (%) is less than 15%. [CV(%) = SD/mean ×100]

Storage Stability: The loss activity rate of this kit is less than 5% within the expiration date under appropriate storage condition.

3. MATERIALS SUPPLIED

Items	Materials	48 Tests	96 Tests
1	Microelisa Stripplate	12×4 Strips	12×8 Strips
2	Standards (6 vials)	0.5ml×6 vials	0.5ml×6 vials
3	Sample Diluent	3.0ml	6.0ml
4	HRP-Conjugate Reagent	5.0ml	10.0ml
5	20×Wash Solution	15ml	25ml
6	Stop Solution	3.0ml	6.0ml
7	Chromogen Solution A	3.0ml	6.0ml
8	Chromogen Solution B	3.0ml	6.0ml
9	Closure Plate Membrane	2	2
10	Sealed Bags	1	1
11	Instruction	1	1

Note: The concentration gradients of Standards are followed by: 8,4,2,1,0.5,0.25 ug/ml.

4. MATERIALS REQUIRED BUT NOT SUPPLIED

1) Microplate reader capable of measuring absorbance at 450 nm.

2) Precision pipettes and Disposable pipette tips.

3) An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

4) Distilled or deionized water.

5) Absorbent paper for blotting the plate.

5. PRECAUTIONS

1) Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.

2) Limited by current skill and knowledge, it is impossible for us to complete the cross-reactivity detection between Human CETP and all the analogues, therefore, cross reaction may still exist.

3) The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. So even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. Please make sure that sufficient samples are available.

4) There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results.

5) Do not remove the plate from the storage bag until needed. Do not substitute reagents from one kit to another. The reagents and the plate are matched for optimal performance. Use only the reagents supplied by manufacturer.

6) Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.

7) Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.

8) Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.

9) The Stop Solution suggested for use with this kit is an acid solution, so please pay enough attention to safety when use it. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

6. SAMPLES COLLECTION AND STORAGE

Serum - Allow samples to clot for two hours at room temperature or overnight at 2°C-8°C before centrifugation for 20 minutes at approximately 1000 × g (or 3000 rpm). Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 20 minutes at 1000 × g (or 3000 rpm) at 2°C-8°C within 30 minutes after collection. Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02mol/L, pH 7.2-7.4). Remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.) with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, centrifugate homogenates for 15 minutes at 1500×g (or 5000 rpm). Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Feces - Collect by sterile tube. Dilute the samples in a certain amount of PBS (0.02mol/L, pH 7.2-7.4). Usually 10mg feces to 100μl PBS. Fully shaking and after 10 minutes' standing, Centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C.

Urine and Body Fluids - Collect by sterile tube. 1000 × g (or 3000 rpm) for approximately 20 minutes. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. When sediments occurred during storage, centrifugation should be performed again. When collecting **Pleuroperitoneal Fluid** and **Cerebrospinal Fluid**, please follow the procedures above-mentioned.

Important Note:

1) We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2) Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤one month) or -80°C(≤two months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.

3) Grossly hemolyzed samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

4) If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

5) Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

6) Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

7) Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

8) The kit can not assay the samples which contain sodium azide(NaN3), because NaN3 will inhibit the activity of horseradish peroxidase (HRP).

7. REAGENT PREPARATION AND STORAGE

Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Wash Solution is stable for one month at 2°C-8°C. Store the kit at 2°C-8°C ! When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. The kit should not be used beyond the expiration date.

8. ASSAY PROCEDURE

1) Prepare all reagents and bring all reagents and samples to room temperature (18°C-25°C) naturally for 30min before starting assay procedure. Do not use hot water baths to thaw samples or reagents. It is recommended that all Standards and samples be added in duplicate to the plate.

2) Set standard wells and testing sample wells, add standard 50µl to each standard well. add sample 50µl to each testing sample well. **Blank well add nothing**.

3) Add 100µl of HRP-conjugate reagent to each well **except the blank well**, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4) Wash the Microtiter Plate 4 times.

Manual Washing - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1×), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. **Note**: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5) Add Chromogen Solution A 50µl and Chromogen Solution B 50µl to **each well** successively. Gently mix and then **protect from light** to incubate for 15 minutes at 37°C.

6) Add 50µl Stop Solution to **each well**. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7) Read the Optical Density (O.D.) at 450 nm using a Microelisa Stripplate reader within 15 minutes.

Important Note::

1) Samples or Reagents Addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and samples, although not required, is recommended. To avoid ontamination, use fresh disposable pipette tips for each transfer to avoid contamination.

2) Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

3) Washing Plate: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

4) Controlling of Reaction Time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

5) Chromogen Solution B is easily contaminated, it should remain colorless or light blue until added to the plate, please protect it from light.

6) The color developed in the wells will turn from blue to yellow after added the Stop Solution. If the color turns green, it indicate the Stop Solution has not mixed thoroughly.

9. CALCULATION OF RESULTS

1) The standard curve is generated by plotting the average O.D. (450 nm) obtained from each Standard on the horizontal (X) axis versus the corresponding each Standard concentration on the vertical (Y) axis.

2) Average the duplicate readings for each standard and sample to subtract average optical density of the Blank.

3) Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain his own standard curve.

4) If the values of samples are higher than the highest concentration of Standards, please dilute the samples with Sample Diluent and repeat to assay the samples. Normally the concentration gradients of Standards cover far more than the range of Human CETP concentrations in normal Human cells, tissue or body fluid, so there is no need to dilute samples when using our kits. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

10. TYPICAL STANDARD CURVE: The following data and curve is solely to demonstrate how to calculate results and should not be taken as an example of best practices of this kit.

Standards (ng/ml):	Blank	31.2	62.5	125	250	500	1000
Mean O.D.(450nm):	0.042	0.137	0.223	0.354	0.601	1.155	2.181
Adjusted:	None	0.095	0.181	0.312	0.559	1.113	2.139