Datasheet for ABIN1113345

alphaHBDH ELISA Kit

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Overview

Quantity:	96 tests
Target:	alphaHBDH
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.156-10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of αHBDH in human serum, plasma and other biological fluids
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit recognizes natural and recombinantHumanαHBDH. No significant cross-reactivity or interference between HumanαHBDH and analogues was observed. Note: Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between HumanαHBDH and all the analogues.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between human alpha-Hydroxybutyrate Dehydrogenase and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Human bFGF and all the

Product Details

	analogues, therefore, cross reaction may still exist.
Sensitivity:	0.094 ng/mL
Components:	Micro ELISA Plate Reference Standard Concentrated Biotinylated Detection Ab Concentrated HRP Conjugate Reference Standard & Sample Diluent Biotinylated Detection Ab Diluent HRP Conjugate Diluent Concentrated Wash Buffer (25×) Substrate Reagent Stop Solutio Plate Sealer Product Description Certificate of Analysis
Material not included:	Microplate reader with 450nm wavelength filter High-precision transferpettor, EP tubes and disposable pipette tips 37*℃ Incubator* Deionized or distilled water Absorbent paper Loading slot for Wash Buffer

Target Details

Target:	alphaHBDH
Alternative Name:	alpha-Hydroxybutyrate Dehydrogenase (alphaHBDH Products)

Application Details

Application Notes:	**ELISA Plate:** The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experiment results. **Add Sample:** The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel

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measure ment is recommended.

Incubation: To prevent evaporation and 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. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.

Washing: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be pat dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.

Reagent Preparation: As the volume of Detection Ab and HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into small pack according to the amount of each assay, keep them at -20°C to -80°C and avoid repeated freezing and thawing.

Reaction Time Control: Please control reaction time strictly following this product description!

Substrate: Substrate Solution is easily contaminated. Please protect it from light.Stop Solution: As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.

Mixing: You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.

Security: Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

Do not use component from different batches of kit(washing buffer and stop solution can be an exception)

To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. Otherwise, the results will be inaccurate!

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Application Details

Comment:	Information on standard material: The formulation of the standard is 0.01 M PBS. The standard contains additives (1 % BSA). Information on reagents: Reagents include 1 M SO_2. Azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials are not used. Information on antibodies: The provided antibodies and their host vary in different kits. All antibodies are affinity purified The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to αHBDH. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for αHBDHand Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain α HBDH, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of αHBDH.You can calculate the concentration of αHBDHin the samples by comparing the OD of the samples to the standard curve. 6th Edition, revised in June, 2015 www.elabscience.com 4
Reagent Preparation:	Bring all reagents to room temperature (18-25°C) before use. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

	Standard: Prepare standard within 15 minutes before use. Reconstitute the Standard with 1.0 mL of Sample Diluent, let it stand for 10 minutes until it dissolved fully. This reconstitution produces a stock solution. Then make serial dilutions as needed (Making serial dilution in the wells directly is not permitted). The Sample Diluent serves as the zero (0). **Biotinylated Detection Ab:** Calculate the required amount before experiment (100μL /well). In actual preparation, you should prepare 100-200μL more. Centrifuge the stock tube before use, dilute the concentrated Biotinylated Detection Ab to the working concentration using Diluent for Biotinylated Detection Ab (1:100). **Concentrated HRP Conjugate:** Calculate the required amount before experiment (100μL /well). In actual preparation you should prepare 100-200μL more. Dilute the Concentrated HRP Conjugate to the working concentration using Diluent for Concentrated HRP Conjugate (1:100). **Substrate Reagent:** As it is sensitive to light and contaminants, so you shouldn't open the vial until you need it! The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent shouldn't be dumped back into the vial again. **Note:** please don't prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.
Sample Collection:	Samples should be clear and transparent and be centrifuged to remove suspended solids. **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000×g. 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 or heparin 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. Hemolysis samples are not suitable for ELISA assay! **Other biological fluids:** Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately. **Tissue homogenates:** You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.02M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate. Note: Samples should be used within 7 days when stored at 2-8°C, otherwise samples must be

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divided and stored at -20°C (≤ 1month) or -80°C (≤ 6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.

Please take the samples to room temperature (18-25°C) without extra heating before performing the assay.

Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure:

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. It's recommended that all samples and standards be assayed in duplicate.

1. Add Sample: Add 100μL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37°C.

2. Biotinylated Detection Ab: Remove the liquid of each well, don'tn'tn't wash. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.

3. Wash: Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

4. HRP Conjugate: Add 100μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.

5. Wash: Repeat the wash process for five times as conducted in step 3.

6. Substrate: Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.

7. Stop: Add 50μLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.

8. OD Measurement: Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm. User should open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

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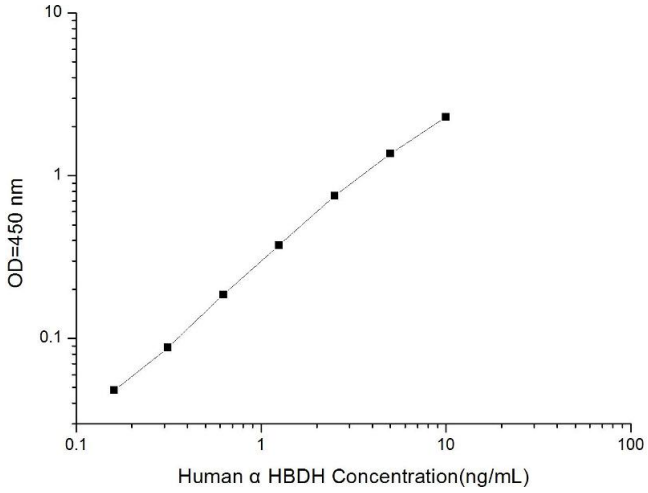
	9. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry
Calculation of Results:	Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value for each standard on the y-axisagainst the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. Substrate Reagent shouldn't be kept at -20 °C (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.
Storage:	4 °C/-20 °C
Storage Comment:	The unopened kit can be stored at 4*°C for 1 month. If the kit is not used within 1 month, store the items separately according to the conditions since the kit is received.*



ELISA

Image 1. Typical standard curve

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