ab287821 – Human Alcohol Dehydrogenase ELISA Kit

For the in vitro quantitative determination of ADH1A in human serum, plasma, tissue homogenates and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab287821

Storage and Stability

The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 X 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)	120 µl	4°C / Avoid light
SABC dilution buffer	10 ml	4°C
TMB substrate	10 ml	4°C / Avoid light
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
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Biotin- detection antibody working solution: Calculate the total volume required of the diluted/working solution: 0.1 ml / well × quantity of wells, with an additional 0.1 - 0.2 ml. Dilute sufficient Biotin- detection antibody with Antibody dilution buffer at 1:100 to make up this calculated volume, and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume required of the diluted/working solution: 0.1 ml / well × quantity of wells, with an additional 0.1 - 0.2 ml. Dilute sufficient SABC with SABC dilution buffer at 1:100 to make up this calculated volume, and mix thoroughly.

Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer into 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 in 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.

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Standard Preparation

1. Reconstitute the lyophilized ADH1A standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 10 ng/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 5 ng/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.
4. Suggested standard points are: 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0 ng/ml.

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum for 2 hours at room temperature, or overnight at 4°C. Centrifuge at approximately 1000 x 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 x g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysed and high cholesterol samples.

Tissue homogenates: Rinse the tissues with ice-cold PBS (10 mM, pH 7.4) to remove excess hemolysed blood thoroughly. Tissue pieces should be weighed and then minced to small pieces, 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 x g to retrieve the supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000 x 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 x g at 4°C. Collect the supernatant and carry out the assay immediately.

Δ Note: *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.*

Assay Procedure

- 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 instructed above.
2. Wash plate 2 times with 1X Wash Solution before proceeding with the assay.
3. Add 100 µl of each standard or sample into appropriate wells. Cover well and incubate for 1.5 hours at 37°C.
4. Remove the cover and discard the plate contents 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 autowasher. 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 within 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 adsorbance results at 450 nm within 20 minutes.

Calculations

Calculate the relative optical density as below:

relative O.D.450 = O.D.450 of each well – O.D.450 of Zero well

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). The Human ADH1A concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Technical Support

For all technical or commercial enquiries please go to:
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