Human dopamine-β-hydroxylase ELISA KIT

Rev.04-2

Catalog no	**BZ-08124100-CPEB**
Size	**96 wells**

User Instruction

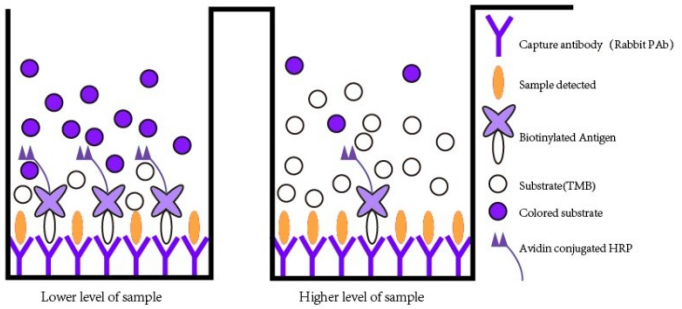
Standard Curve Range	3 ng/ml – 900 ng/ml
Sensitivity	1.26 ng/ml
Repeatability	Intra-Assay: CV < 10%; Inter-Assay: CV < 12%
Storage	Keep the **whole kit** before use in: • -20°C for 12 months validity • 2-8°C for 6 months validity Avoid repeated freeze–thaw cycles. Do not use the kit beyond its expiration date.

Specificity

This Competitive ELISA kit is for the quantification of Human dopamine-beta-hydroxylase (also known as DBH) in serum, plasma, cell culture supernatants, urine, tissue homogenates or other biological fluids. Note: This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.

Assay Principle

This kit is a Enzyme-Linked Immunosorbent Assay (ELISA). Add samples to the pre-coated plate. Then add biotinylated antigen. The antigens in the samples compete with the biotinylated antigen to bind to the capture antibody and incubate. Unbound antigen is washed away during a washing step. An avidin-HRP is then added and then incubate. Unbound avidin-HRP is washed away during a washing step. TMB Substrate is then added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of DBH in the sample. The concentration of DBH in the sample is then determined by comparing the O.D. of the samples to the standard curve.



Storage Directions and Kit Components

Keep the **whole kit** before use in:

- -20°C for 12 months validity
- 2-8°C for 6 months validity

After opening handling, store the items separately according to the following directions:

Components	96 Wells	Storage Directions After Opening Handling
Pre-Coated Microplate	12 x 8 wells	2-8°C (1 week) / -20°C (1 months)
Standard/Sample Diluent	6 ml	
Human DBH Standard, lyophilized	2 vials	
Avidin HRP Diluent	5.9 ml	
Avidin-HRP Concentrate	100 µl	
Biotinylated Antigen Diluent	6 ml	
Biotinylated Antigen, lyophilized	1 vial	
Stop Solution	6 ml	
Wash Buffer Concentrate (25x)	20 ml	
Substrate Solution A	6 ml	
Substrate Solution B	6 ml	2-8°C (1 week) / -20°C (1 months) (light sensitive, avoid light)
Plate Covers	2	RT
Instruction manual	1	

Notes:

- If the whole kit is stored at -20°C, place the kit at 2-8°C the day before the experiment.
- After receiving the kit, the plates can be disassembled to single strips. Please use it in batches on demands.

Material Required But Not Supplied

- 37°C±0.5°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- Prior to running the assay, the kit and sample should be warmed naturally to room temperature 30 minutes.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- There are two vials of standard in the kit for users, please cover the other unused vial and keep refrigerated.
- Do not allow wells to become dry during the assay procedure.
- This instruction should be strictly followed in the experiment.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.

- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 20 minutes at 2000-3000 RPM within 10-20 minutes of collection. Collect the supernatant without sediment.

Urine/Ascites/Cerebrospinal fluid Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatant without sediment.

Cell Culture Supernatant Collect by sterile tubes when examining secrete components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatant without sediment.

Tissue Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4℃ to get the supernatant.

Note

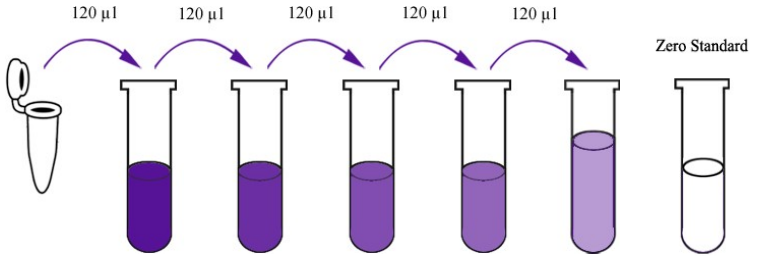
- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the sample used in the whole test.
- Samples to be used within 5 days should be stored at 2-8℃. Samples should be aliquoted or must be stored at -20℃ within 1 month or -80℃ within 3 months. Avoid repeated freeze thaw cycles.

- Samples should be brought to room temperature before starting the assay.
- Samples containing NaN3 cannot be tested as it inhibits the activity of HRP.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Sample can't be diluted with this kit. Owing to the material we use to prepare the kit, the sample matrix interference may falsely depress the specificity and accuracy of the assay.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Standard** Reconstitute one vial of standard with 150 µl of Standard/Sample Diluent to generate a 960 ng/ml standard stock solution which should be used within 24 hours. Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution 1: 2 with diluent to produce 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, and 2ng/mL solutions. Add Standard/Sample diluent only as the zero standard (0 ng/mL).



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
960 ng/ml	480 ng/ml	240 ng/ml	120 ng/ml	60 ng/ml	30 ng/ml

- **Biotinylated Antigen** Briefly centrifuge the biotinylated antigen vial then add 1 ml Biotinylated Antigen Diluent to mix well. And then pipette all this solution back into the Biotinylated Antigen diluent vial to mix well and generate a 6 ml stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions.
- **Avidin-HRP Concentrate** Briefly low- speed centrifuge the avidin-HRP Concentrates solution and then pipette all avidin-HRP into the Avidin HRP Diluent vial. Mix well to generate a 6 ml stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions.
- **Wash Buffer Concentrate 25x** Dilute 20 ml of concentrated wash buffer with 480 ml double distilled water to prepare 500 ml of wash buffer. If crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8℃ for up to one month.
3. Blank wells: Only add substrate solution A, substrate solution B and Stop solution as blank control.
4. Add 50 µl diluted standard to standard well, add 50ul sample (Sample recommended dilution: 2-5 times when necessary) to the sample well, and add 50 µl biotinylated antigen to each well. Mix well. Cover the plate with a sealer and incubate for 60 minutes at 37℃.
5. Remove the sealer and the liquid in the well, wash five times with 300 µl wash buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to complete remove liquid. For automated washing, aspirate all wells and wash 5 times with wash buffer. Blot the plate on absorbent material.
6. Add 50 µl avidin-HRP to the standard well and sample well, cover the plate with a sealer and incubate for 60 minutes at 37℃.
7. Remove the sealer and wash as described above.
8. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37℃ in the dark.
9. Add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.
10. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

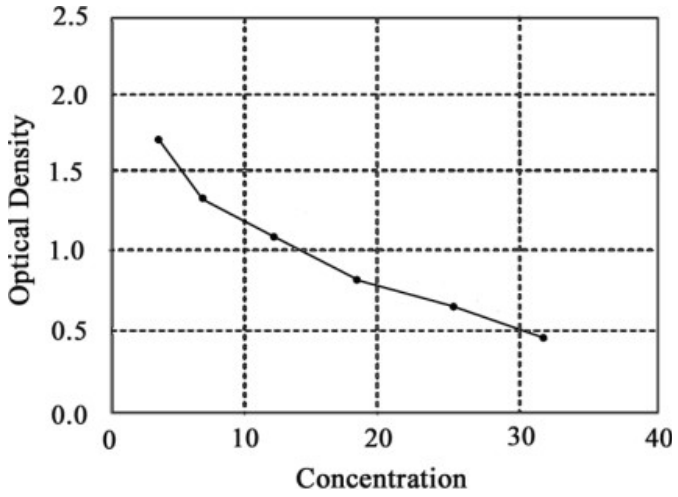
Calculation of Result

Average the duplicate readings for each standard, control, and sample. Create a standard curve by plotting the mean absorbance for each standard on the Y-axis against the target antigen concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the X axis versus the O.D. of the standards on the Y axis and the best fit line can be determined by regression analysis. The linear equation (X = Y + Calibration Value) can be used to calculate the standard curve where X is the log of the concentration of the standard and Y is the OD value of the standard. If samples have been diluted (2-5 times is recommended), the concentration read from the standard curve must be multiplied by the dilution factor.

Concentration	Blank	S5	S4	S3	S2	S1	S0
OD value	VB	V5	V4	V3	V2	V1	V0
Calibration value	VB-VB	V5-VB	V4-VB	V3-VB	V2-VB	V1-VB	V0-VB

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Summary

1. Prepare all reagents, samples, and standards.
2. Add samples, standards, and biotinylated antigen. Incubate for 60 minutes at 37°C.
3. Aspirate and wash 5 times.
4. Add avidin-HRP and incubate for 60 minutes at 37°C.
5. Aspirate and wash 5 times.
6. Add substrate solution A and substrate solution B and incubate in the dark for 10 minutes at 37°C.
7. Add stop solution
8. Read the OD value within 10 minutes at 450 nm.

Shipping Direction - Temperature

Maintain temperature between 2°C to 8°C during transportation in unopened packaging.

Troubleshooting

Possible Case	Solution
High Background	
• Improper washing • Substrate was contaminated • Non-specific binding of antibody • Plate are not be sealing incompletely • Incorrect incubation temperature • Substrate exposed to light prior to use • Contaminated wash buffer	• Increasing duration of soaking steps • Replace. Substrate should be clean and avoid crossed contamination by using the sealer • Replace another purified antibody or blocking buffer • Make sure to follow the instruction strictly • Incubate at room temperature • Keep substrate in a dark place • Use a clean buffers and sterile filter
Weak Signal	
• Improper washing • Incorrect incubation temperature • Antibody are not enough • Reagent are contaminated • Pipette are not clean	• Increasing duration of soaking steps • Incubate at room temperature • Increase the concentration of the antibody • Use new one • Pipette should be clean
No Signal	
• Reagent are contaminated • Sample prepared incorrectly • Antibody are not enough • Wash buffer contains sodium azide • HRP was not added	• Use new one • Make sure the sample workable/dilution • Increase the antibody concentration • Use a new wash buffer and avoid sodium azide in it • Add HRP according to the instruction
Poor Precision	
• Imprecise/ inaccurate pipetting • Incomplete washing of the wells	• Check/ calibrate pipettes Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.

This product is for research use only, not for use in diagnosis procedures.
It's highly recommended to read this instruction entirely before use.

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