

**User's Manual**

# Human L- Dihydroxyphenylalanine ELISA Kit

**REF****DEIA-NS2502-1**

96T

**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

This sandwich kit is for the accurate quantitative detection of Human L-Dihydroxyphenylalanine (also known as L-DOPA) in serum, plasma, cell culture supernates, ascites, tissue homogenates or other biological fluids.

### Principles of Testing

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human L-DOPA antibody. L-DOPA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human L-DOPA Antibody is added and binds to L-DOPA in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated L-DOPA antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human L-DOPA. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

### Reagents And Materials Provided

1. Standard Solution (3200ng/L): 0.5ml × 1
2. Pre-coated ELISA Plate: 12 \* 8 well strips × 1
3. Standard Diluent: 3ml × 1
4. Streptavidin-HRP: 6ml × 1
5. Stop Solution: 6ml × 1
6. Substrate Solution A: 6ml × 1
7. Substrate Solution B: 6ml × 1
8. Wash Buffer Concentrate (25×): 20ml × 1
9. Biotinylated Human L-DOPA Antibody: 1ml × 1
10. User Instruction: 1
11. Plate Sealer: 2 pics
12. Zipper bag: 1 pic

### Materials Required But Not Supplied

1. 37°C±0.5°C incubator
2. Absorbent paper
3. Precision pipettes and disposable pipette tips
4. Clean tubes
5. Deionized or distilled water
6. Microplate reader with 450 ± 10nm wavelength filter

## Storage

Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

## Specimen Collection And Preparation

1. **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.
2. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.
3. **Urine/Ascites/ Cerebrospinal fluid:** Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.
4. **Cell culture supernatant:** Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, 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 20 minutes. Collect the supernatant without sediment.
5. **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 5 minutes at 5000×g to get the supernatant.

### Note

1. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for their particular experiments.
2. Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.
3. Samples should be brought to room temperature before starting the assay.
4. Centrifuge to collect sample before use.
5. Samples containing NaN<sub>3</sub> can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).
6. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
7. Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

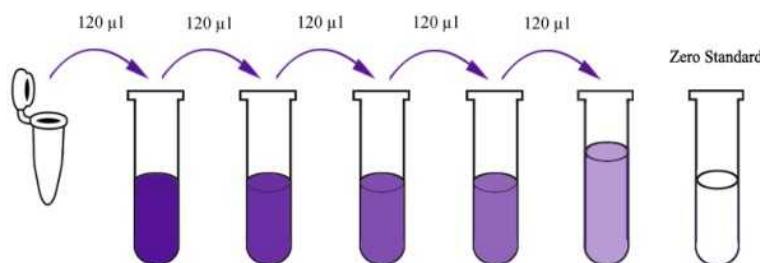
*\*Sample can't be diluted with this kit. Owing to the the material we use to prepare the kit, the sample matrix interference may falsely depress the specificity and accuracy of the assay.*

## Reagent Preparation

1. All reagents should be brought to room temperature before use.

2. Standard: Reconstitute the 120 $\mu$ l of the standard (3200ng/L) with 120 $\mu$ l of standard diluent to generate a 1600ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1600ng/L) 1:2 with standard diluent to produce 800ng/L, 400ng/L, 200ng/L and 100ng/L solutions. Standard diluent serves as the zero standard(0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

1600ng/L	Standard No.5	120 $\mu$ l Original Standard + 120 $\mu$ l Standard Diluent
800ng/L	Standard No.4	120 $\mu$ l Standard No.5 + 120 $\mu$ l Standard Diluent
400ng/L	Standard No.3	120 $\mu$ l Standard No.4 + 120 $\mu$ l Standard Diluent
200ng/L	Standard No.2	120 $\mu$ l Standard No.3 + 120 $\mu$ l Standard Diluent
100ng/L	Standard No.1	120 $\mu$ l Standard No.2 + 120 $\mu$ l Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
3200ng/L	1600ng/L	800ng/L	400ng/L	200ng/L	100ng/L

3. Wash Buffer: Dilute 20ml of Wash Buffer Concentrate 25 $\times$  into deionized or distilled water to yield 500 ml of 1 $\times$  Wash Buffer. If crystals have formed in the concentrate, mix 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°C.
3. Add 50 $\mu$ l standard to standard well.

**Note:** Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 40 $\mu$ l sample to sample wells and then add 10 $\mu$ l anti-L-DOPA antibody to sample wells, then add 50 $\mu$ l streptavidin-HRP to sample wells and standard wells ( Not blank control well ). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times

with wash buffer. Blot the plate onto paper towels or other absorbent material.

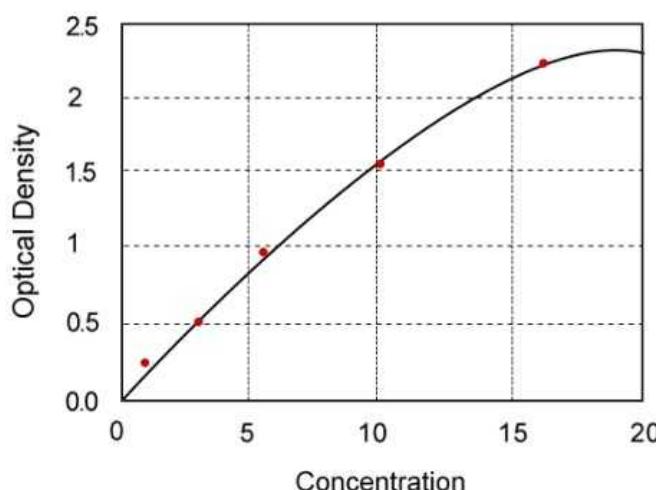
6. Add 50 $\mu$ l substrate solution A to each well and then add 50 $\mu$ l substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 $\mu$ l Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the 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

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

## Typical Standard Curve

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



## Precision

**Intra-Assay Precision (Precision within an assay)** Three samples of known concentration were tested on one plate to assess intra-assay precision.

**Inter-Assay Precision (Precision between assays)** Three samples of known concentration were tested in separate assays to assess inter-assay precision.

$$CV(\%) = \frac{SD}{mean} \times 100\%$$

Intra-Assay: CV<8%

Inter-Assay: CV<10%

## Sensitivity

5.12ng/L

## Linearity

10-3000ng/L

## Precautions

1. Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
2. This instruction must be strictly followed in the experiment.
3. 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.
4. Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
5. Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
6. Avoid using the reagents from different batches together.
7. Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
8. 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.
9. The kit should not be used beyond the expiration date.