Rat Dopamine (DA) ELISA Research Kit

Product code: 248683

Product Introduction

Dopamine (DA), also known as 3,4-dihydroxyphenethylamine, is a neurochemical in the brain. Its chemical formula is $C_6H_3(OH)_2$-CH_2-CH_2-NH_2, and its formal chemical name is 4-(2-aminoethyl)benzene-1,2-diol.

Dopamine is a neurotransmitter in the catecholamine and phenethylamine family and accounts for about 80% of the catecholamine content in the brain. It is synthesized from its precursor L-DOPA by removal of a carboxyl group. L-DOPA is synthesized in the brain and kidneys. Dopamine is also synthesized in plants and most animals.

This kit uses a competitive enzyme-linked immunosorbent assay (ELISA). Microwells are pre-coated with rat dopamine (DA) antigen. Samples, standards, biotin-labeled antibody, and HRP enzyme conjugate are added in sequence. After incubation and washing, TMB substrate is used for color development. Under HRP catalysis, TMB turns blue and then becomes yellow after acid is added. The color intensity is negatively correlated with the rat dopamine (DA) concentration in the sample. Measure absorbance at 450 nm and calculate sample concentration.

- Sensitivity: 7.2 pg/mL
- Detection range: 15.6-1000 pg/mL
- Specificity: Rat DA in samples can be detected, with no significant cross-reactivity.

Package Contents

Item Code	Component	48T	96T
248683.1	Pre-coated 96 well microplate	8 wells × 6 strips	8 wells × 12 strips
248683.2	Standard	1 vial	2 vials
248683.3	Universal Diluent	20 mL × 1	20 mL × 2
248683.4	Concentrated Biotin-Antibody (100×)	30 μL	60 μL
248683.5	Concentrated Enzyme Conjugate (100×)	60 μL	120 μL
248683.6	Wash Solution (20×)	10 mL × 1	10 mL × 2
248683.7	Substrate TMB	5 mL	10 mL
248683.8	Stop Solution	3 mL	6 mL
248683.9	Plate Sealer	4 sheets	4 sheets
248683.m	Manual	1 copy	1 copy

Quality Standards and Safety Instructions

Material or Packaging	Quality Standards	Main Toxicity
Pre-coated 96 well microplate	—	—
Standard	—	—
Universal Diluent	—	—
Concentrated Biotin-Antibody (100×)	—	—
Concentrated Enzyme Conjugate (100×)	—	—
Wash Buffer (20×)	—	—
Substrate TMB	—	—
Stop Solution	—	—
Plate Sealer	—	—

Transportation and Storage

- Transportation: Transport with ice packs.
- Storage: Store at 2-8°C for 180 days.

Sample Handling

> The kit detection range is not the same as the analyte concentration range in the sample. Estimate the expected analyte concentration from relevant literature before testing, then confirm the actual concentration with a preliminary experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.
>
> If the sample type is not listed below, perform a preliminary experiment to verify suitability.

General Requirements

- Samples should be clear and transparent. Remove suspended matter by centrifugation.
- If testing within 1 week of collection, store samples at 4°C.
- If testing is delayed, aliquot into single-use portions and store at -20°C for up to 1 month or at -80°C for up to 6 months.
- Avoid repeated freeze-thaw cycles.
- Hemolyzed specimens are not suitable for this test.

Sample Types

1. **Serum:** Let whole blood collected in a serum separator tube stand at room temperature for 2 hours or at 4°C overnight. Centrifuge at 1000×g for 20 minutes and collect the supernatant. Store at -20°C or -80°C if needed. Avoid repeated freeze-thaw cycles.
2. **Plasma:** Use EDTA or heparin as anticoagulant. Within 30 minutes after collection, keep at 2-8°C and centrifuge at 1000×g for 15 minutes. Collect the supernatant for testing or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
3. **Tissue homogenate:** Rinse tissue with pre-chilled PBS (0.01 M, pH 7.4) to remove residual blood. Weigh and mince the tissue. Add PBS at a typical weight-to-volume ratio of 1:9, for example 1 g tissue with 9 mL PBS. Record the exact volume used. Homogenize thoroughly on ice in a glass homogenizer. Adding protease inhibitors to PBS is recommended. To further lyse cells, use ultrasonic disruption or repeated freeze-thaw cycles. Centrifuge at 5000×g for 5-10 minutes and collect the supernatant.
4. **Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes. Collect the supernatant for testing or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
5. **Other biological samples:** Centrifuge at 1000×g for 20 minutes and collect the supernatant for testing.

Test Procedure

Reagent Preparation

1. Remove the kit from the refrigerator 10 minutes before use and allow it to equilibrate to room temperature.
2. **Standard working solutions:** Add 1 mL universal diluent to the lyophilized standard. Let stand for 15 minutes until fully dissolved, then mix gently to obtain a 1000 pg/mL standard. Prepare standards at 1000, 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/mL.
3. **Serial dilution method:** Prepare 7 tubes, each containing 500 µL universal diluent. Add 500 µL of the 1000 pg/mL standard to the first tube and mix well to prepare 500 pg/mL. Repeat sequentially to prepare 250, 125, 62.5, 31.2, and 15.6 pg/mL standards. Use universal diluent as the 0 pg/mL blank.
4. **Biotin-antibody working solution:** 15 minutes before use, centrifuge the 100× concentrated biotin-antibody at 1000×g for 1 minute. Dilute to 1× with universal diluent, for example 10 µL concentrate + 990 µL diluent. Use the working solution on the same day.
5. **Enzyme conjugate working solution:** 15 minutes before use, centrifuge the 100× concentrated enzyme conjugate at 1000×g for 1 minute. Dilute to 1× with universal diluent, for example 10 µL concentrate + 990 µL diluent. Use the working solution on the same day.
6. **1× wash solution:** Add 10 mL of 20× wash solution to 190 mL distilled water. Crystals may form during cold storage; this is normal. Allow the solution to return to room temperature and dissolve completely before use.

Assay Procedure

1. After 10 minutes at room temperature, remove the required strips from the foil pouch. Place unused strips in a resealable bag, seal, and return to 4°C.
2. Add 50 μL of standards or samples to the appropriate wells. Add 50 μL universal diluent to blank wells. Immediately add 50 μL biotin-antibody working solution to each well. Cover with a plate sealer and incubate at 37°C for 1 hour.
3. Wash the plate: discard the liquid, add 300 μL 1× wash solution to each well, let stand for 1 minute, remove the wash solution, and blot dry on absorbent paper. Repeat 3 times. A plate washer may also be used.
4. Add 100 μL enzyme conjugate working solution to each well. Cover with a plate sealer and incubate at 37°C for 30 minutes.
5. Wash the plate again using the same method as step 3, for a total of 5 washes.
6. Add 90 μL TMB substrate to each well. Cover with a plate sealer and incubate at 37°C in the dark for 15 minutes.
7. Add 50 μL stop solution to each well. Immediately read the OD value at 450 nm.

To reduce matrix effects, it is recommended to dilute samples with universal diluent before loading the plate. When calculating the final concentration, multiply by the corresponding dilution factor. Standards and samples are recommended to be run in duplicate.

Result Interpretation

1. Calculate the mean OD values of duplicate wells for standards and samples.
2. Use concentration as the x-axis and OD value as the y-axis. Plot a four-parameter logistic standard curve on double-logarithmic graph paper. Exclude the blank group when plotting the curve.
3. If a sample OD value exceeds the upper limit of the standard curve, dilute the sample appropriately, retest, and multiply the result by the dilution factor.

Reference Standard Curve Data

The following data are for reference only. A standard curve must be established for each experiment.

Concentration (pg/mL)	1000	500	250	125	62.5	31.2	15.6	0
OD Value	0.216	0.298	0.417	0.669	0.955	1.405	1.79	1.865

Precautions

1. Incubate strictly according to the specified time and temperature. All reagents must reach room temperature, 20-25°C, before use. Return reagents to refrigeration immediately after use.
2. Incorrect plate washing may cause inaccurate results. Before adding substrate, remove liquid from wells as completely as possible. Do not allow microwells to dry out during incubation.
3. Remove residual liquid and fingerprints from the bottom of the plate before reading, as these may affect OD values.
4. The substrate chromogenic solution should be colorless or very light in color. Do not use substrate solution that has already turned blue.
5. Avoid cross-contamination of reagents and specimens.
6. Avoid direct exposure to strong light during storage and incubation.
7. No reaction reagent may come into contact with bleach solvent or strong bleach vapors. Bleaching components will destroy the biological activity of the reagents.
8. Do not use expired products. Do not mix components with different item numbers or batch numbers.
9. Recombinant proteins from sources outside this kit may not match the antibodies in this kit and may not be recognized.
10. If disease transmission is possible, handle all samples according to the prescribed procedures for samples and testing devices.

Visual Reference