Product Manual

Dopamine ELISA Kit

Catalog #: ENZ-KIT188-0001

96-Well Kit

For the latest product information, including support documentation, visit us online:
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Technical Support (US): 800-942-0430
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Please read entire booklet before proceeding with the assay.

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INTRODUCTION

Dopamine is an organic chemical of the catecholamine and phenethylamine families that plays several important roles in the brain and body. In the brain, dopamine functions as a neurotransmitter. Outside the central nervous system, dopamine functions in several parts of the peripheral nervous system as a local chemical messenger. Enzo's Dopamine ELISA kit is a competitive ELISA assay for the quantitative measurement of dopamine in serum, plasma, and cell culture supernatants. The density of color is inversely proportional to the amount of dopamine captured from the samples.

APPLICATION

The ELISA is used for quantitative determination of dopamine. This kit is intended for **research use only.**

Detection range: 1.56 – 100 ng/mL

Sensitivity: < 0.938 ng/mL

MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

Protect substrate from prolonged exposure to light.

Stop solution is caustic. Keep tightly capped.

1. **Micro ELISA Plate, One Plate of 96 Wells, 8 X 12 Strips**
   A plate using break-apart strips coated with antigen.
2. **Lyophilized Standard, 2 Vials**
3. **Sample/Standard Dilution Buffer. 20 mL**
4. **Biotin-Detection Antibody (concentrated), 60 µL**
   Antibody specific to biotin.
5. **Antibody Dilution Buffer, 10 mL**
   Buffer used to dilute the biotin antibody concentrate.
6. **HRP-Streptavidin Conjugate (SABC), 120 µL**
   Protect from light.
7. **SABC Dilution Buffer, 10 mL**
8. **TMB Substrate, 10 mL**
   Protect from light.
9. **Stop Solution, 10 mL**
   A 1N solution of hydrochloric acid in water.
10. **Wash Buffer (25X), 30 mL**
11. **Plate Sealers, 5**

STORAGE

All kit components are stable at 4°C until 6 months from the date of receipt.

OTHER MATERIALS NEEDED

1. Microplate reader capable of measuring absorbance at 450 nm
2. 37° C incubator
3. Precision pipettes with disposable tips
4. Distilled or deionized water
5. Clean Eppendorf tubes for preparing standards and sample dilutions
6. Absorbent paper

REAGENT PREPARATION

Sample handling procedures should be completed prior to 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 the tubes.

1. **Biotin-Detection Antibody Working Solution**

   Calculate the total volume of the working solution: 0.05 mL/well x quantity of wells with additional 0.1 – 0.2 mL of the total volume. Dilute the biotin-detection antibody with antibody dilution buffer at 1:100 and mix thoroughly.

2. **HRP-Streptavidin Conjugate (SABC)**

   Calculate the total volume of the working solution: 0.1 mL/well x quantity of wells with additional 0.1 – 0.2 mL of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

3. **Wash Buffer**

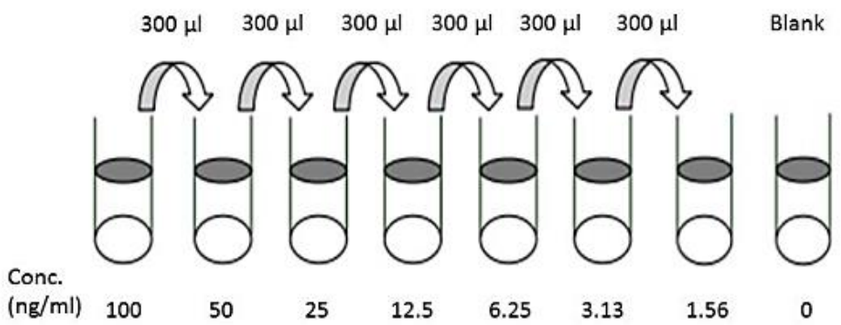
   Dilute 30 mL of concentrated wash buffer into 750 mL of wash buffer with deionized or distilled water. Put unused solution back at 4° C. If crystals have formed in the concentrate, warm it in a 40° C water bath and mix gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

STANDARD PREPARATION

Use 100 ng/mL standard stock solution within 2 hours of reconstitution.

1. Reconstitute the lyophilized dopamine standard by adding 1 mL of standard/sample dilution buffer to make the 100 ng/mL standard stock solution.
2. Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
3. Prepare 0.6 mL of 50 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. Suggested standard points are: 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0 ng/mL.

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.



SAMPLE PREPARATION

1. **Serum**

   Coagulate the serum for 2 hours at room temperature or overnight at 4° C. Centrifuge at approximately 1000 x g for 20 minutes. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

2. **Plasma**

   Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 minutes of collection. To eliminate the platelet effect, perform further centrifugation for 10 minutes at 2-8°C at 10000 x g. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.

3. **Tissue Homogenates**

   Rinse the tissues with ice-cold PBS (0.01 M, pH = 7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. It is recommended to add protease inhibitor to the PBS.) with a glass homogenizer on ice. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates should then be centrifuged for 5 minutes at 5000 x g to retrieve the supernatant.

4. **Cell Culture Supernatants**
   Centrifuge supernatant at 1000 x g, 2-8° C, for 20 minutes to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately, or aliquot and store at -20°C.
5. **Other Biological Fluids**
   Centrifuge samples for 20 min at 1000 x g at 4°C. Collect the supernatant and carry out the assay immediately.

End user 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 into the optimal detection range of the kit.

ASSAY PROCEDURE

Bring all reagents to room temperature 30 minutes prior to the assay.

All standards and samples should be run at least in duplicate.

A standard curve should be run for each assay.

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

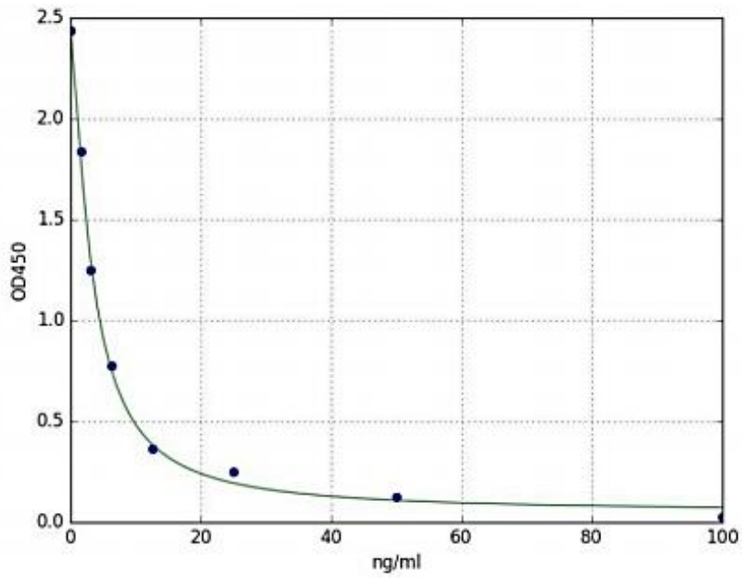
1. Prepare all reagents, samples, and standards as instructed.
2. Wash plate 2 times with 1X Wash Solution before adding standard, sample and control wells.
3. Add 50 µL of each standards or samples into appropriate wells.
4. Immediately add 50 µL of biotin-detection antibody working solution to each well. Cover with the plate sealer, and gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C.
5. Discard the solution and wash 3 times with 1X wash solution. Wash by filling each well with wash buffer (350 µL) using a multichannel pipette or autowasher. Let soak for 1-2 minutes, 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 material.
6. Add 0.1 mL of SABC working solution into each well, cover the plate and incubate at 37ºC for 30 minutes.
7. Discard the solution and wash 5 times with 1X Wash Solution.
8. Add 90 µL of TMB substrate into each well, cover the plate, and incubate at 37 ºC in the dark for 15-20 min. The shades of blue should be seen in the first 3-4 wells by the end of the incubation period.
9. Add 50 µL of stop solution to each well. Read results at 450 nm within 20 minutes.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve must not be used to calculate dopamine concentrations; each user must run a standard curve for each assay.



NOTES

Global Headquarters
Enzo Life Sciences Inc.
10 Executive Blvd
Farmingdale, NY 11735
(p) 1-800-942-0430
(f) 1-631-694-7501
(e) info-usa@enzolifesciences.com

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
(p) +41/0 61 926 89 89
(f) +41/0 61 926 89 79
(e) info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com

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