General Dopamine ELISA Kit (DA)

Catalog NO.: RK00642

version: 2.0

This package insert must be read in its entirety before using this product

Introduction

This ELISA kit used for quantitative determination of DA in serum, plasma, and other biological fluids. For research use only, and it is highly recommended to read thoroughly of this manual before using the product.

Principle of the Assay

Competition method inhibition enzyme immunoassay technique was used. The DA coated microplate is made of solid phase carrier, and the sample to be tested, horseradish peroxidase labeled DA and anti-DA antibody are added to the coated microwell to form the coated secondary antibody-anti-DA antibody-DA (HRP) complex. The amount of labeled DA is inversely proportional to the amount of DA in the sample. After color development, the absorbance value (OD value) was measured in the microplate reader, and the DA content in the sample to be measured was calculated by fitting the concentration-absorbance curve by computer or drawing.

Material Provided & Storage Conditions

Unopened kits can be stored at 2-8°C for 1 year, and opened products must be used within 1 month.

Part	Size	Cat. No.	Storage of opened/reconstituted material
General DA Microwell Plate Coated	8×12	RM04933	Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8℃ for 1 month.
General DA Standard	6 ×1 mL	RM04934	It is not recommended to use again after redissolving.
General DA Antibody	1 x 6 mL	RM04935	Store at 2-8℃ for 1 month *
General DA HRP-Conjugate Antigen	1 x 6 mL	RM04936	Store at 2-8℃ for 1 month *

Wash Buffer(20x)	1 ×15 mL	RM00026	Store at 2-8°C for 1 month *
TMB Substrate A	1 ×7 mL	RM00027	
TMB Substrate B	1 ×7 mL	RM00027	
Stop Solution	1 ×7 mL	RM00028	
Plate Sealers	4 Strips		
Specification	1		

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

*** FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
4. Stop Solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
8. Mix the sample and all components in the kits adequately and use clean plastic container to prepare all diluents.
9. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in

accordance with the requirement of the specification.

10. Reuse of dissolved standard is not recommended.
11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. This assay is designed to eliminate interference by other factors present in biological samples.
17. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
18. The 48T kit is also suitable for the specification.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Other biological fluids: Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatants and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Note: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and

hyperlipidemia sample for serum and plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Standard: Set concentration according to the following below:

Standard	S5	S4	S3	S2	S1	S0
ng/mL	200	37.5	10	2.5	0.5	0

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The washing solution was diluted at 1:20 with double steam or deionized water before use.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Set the blank hole.50 μ L of standard sample / sample was added to the blank wells. Then 50 μ L of enzyme-labeled antigen is added to each well, (Note: do not add to the blank!) Then 50 μ L of antibody was added to each well in the same order. Stir well and cover with the provided sealing plate film. It was incubated for 1 hour at 37℃.
4. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
5. Add wash buffer 250 μ L/well, aspirate each well after holding 1-2 minutes, repeating the process four times for a total of five washes.
6. Add 50 μL of TMB Substrate A and 50 μL of TMB Substrate

B to each well,mix well. Incubate for 15-20 minutes at 37°C. Protect from light.

7. Add Stop Solution (50 μ L/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may cause higher value and less accurate result.

Assay Procedure Summary

Prepare the standard and reagents

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Add 50μL of standards or test samples to each well

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Add 50μL Working HRP-Conjugate Antigen then 50 μL Antibody

Incubate for 1hours at 37℃, then wash five times

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Add 50 μL of TMB Substrate A and 50 μL of TMB Substrate B

Incubate for 15-20 min at 37℃ under dark condition

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Add 50μL Stop Solution

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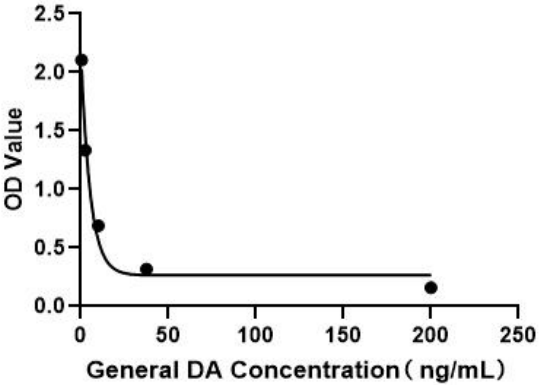
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the DA concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of DA assayed.

Detection Range

0.5-200 ng/mL

Sensitivity

The minimum detectable dose (MDD) of DA typically less than 0.2 ng//mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of DA .

No significant cross-reactivity or interference between DA and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between DA and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-plate Precision

3 samples with low, middle and high level DA were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level DA were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

Recovery

Matrices listed below were spiked with certain level of DA and the recovery rates were calculated by comparing the measured value to the expected amount of DA in samples.

Sample	Average Recovery（%）	Range（%）
Cell Culture Media(n=5)	90	85-98
Serum(n=5)	95	94-100

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of DA and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media(n=5)	Serum(n=5)
1:2	Average of Expected（%）	107	103
	Range（%）	98-113	95-109
1:4	Average of Expected（%）	100	99
	Range（%）	93-103	97-102
1:8	Average of Expected（%）	95	88
	Range（%）	80-109	88-96
1:16	Average of Expected（%）	93	85
	Range（%）	89-100	83-89

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.

	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

*For research purposes only. Not for therapeutic or diagnostic purposes.