



# **Rat Dopamine (DA) ELISA Kit**

**Catalogue Number: RG20111**

**(96Tests)**

**Store all reagents at 2-8°C**

**Collect sample: serum or blood plasma**

**Assay range : 6pg/mL -650pg/mL**

**FOR LABORATORY RESEARCH USE ONLY.**

**NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!**

**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE**

**BEGINNING!**

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## **INTENDED USE**

This DA ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of DA in the sample, this DA ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus DA concentration. The concentration of DA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## **PRINCIPLE OF THE ASSAY**

The kit assay DA level in the sample, use Purified antibody to coat microtiter plate wells, make solid-phase antibody, Samples which including standards of known concentrations and unknowns are pipetted into coated microtiter wells, after Incubating ,add Biotinylated anti-IgG, and Combined Streptavidin-HRP, become antibody – antigen - enzyme- antibody complex, after washing Completely, Add TMB

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substrate solution, TMB Chromogen Solution Becomes blue color At HRP enzyme-catalyzed, And at the effect of acid the color finally become yellow, The intensity of this coloured product is directly proportional to the concentration of DA present in the samples. measure the optical densit (OD) at 450 nm with microtiter plate reader, calculate DA concentration by standard curve.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1. MICROTITER PLATE 96 wells
2. Biotinylated anti-IgG 6.0 mL 1 tube
3. STANDARD(800pg/ml) 0.5ml 1 tube
4. STREPTAVIDIN-HRP 6.0 mL 1 tube
5. STANDARD DILUENT 1.5ml 1 tube
6. Chromogen Solution A 6.0 mL 1 vial
7. Chromogen Solution B 6.0 mL 1 vial
8. STOP SOLUTION 6.0 mL 1 vial
9. WASH SOLUTION x30 20 mL 1 vial

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## 10. Instruction 1

### **SAMPLE COLLECTION AND STORAGE**

Serum- Use a serum separator tube(SST) and allow samples to clot for 30minutes before centrifugation for 15minutes at approximately 1000 x g.Remove serum and assay immediately or aliquot and store samples at -20 °C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant.Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection.Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must stored at -20°C(≤2months) or -80°C(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles .When performing the assay slowly bring samples to room temperature.



DO NOT USE HEAT-TREATED SPECIMENS.

## **MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 ml to 1 ml volumes. .
3. Adjustable 10ml -100ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
6. Absorbent paper.
7. 37°C incubator.
8. Distilled or deionized water.
9. Data analysis and graphing software..
10. Tubes to prepare standard or sample dilutions.

## **PRECAUTIONS**

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal

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performance. Use only the reagents supplied by manufacturer.

2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.



9. All samples should be disposed of in a manner that will inactivate viruses.
10. Solid Waste: Autoclave 60 min. at 121°C.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
13. Chromogen Solution B contains 20% acetone, keep this reagent away from sources of heat or flame.
14. Remove all kit reagents from refrigerator and allow them to reach room temperature ( 20-25°C).

## **REAGENT PREPARATION**

Standard -The Kit provides a stock standard ( 800pg/ml ) . Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions. Pipette 150µL of Standard Dilution into each tube. (total 5 tubes) Use the 150µL of stock solution to produce a 2-fold dilution series (including **400pg/mL, 200pg/mL, 100pg/mL, 50pg/mL,**

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**25pg/mL**). Mix each tube thoroughly before the next transfer.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20mL Wash Buffer Concentrate into deionized or distilled water to prepare 600mL of Wash Buffer.

## **ASSAY PROCEDURE**

Prepare all Standards before starting assay procedure (see Preparation Reagents). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.

1. Determine the number of microwell strips required to test the desired number of samples, Each sample, standard and blank should be assayed in duplicate.
2. add sample: Set blank wells separately (blank comparison wells don't add sample and ELISA reagent, other each step operation is same). Add 50 $\mu$ L of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate, and Gently mix. Incubate for 45 min at 37°C





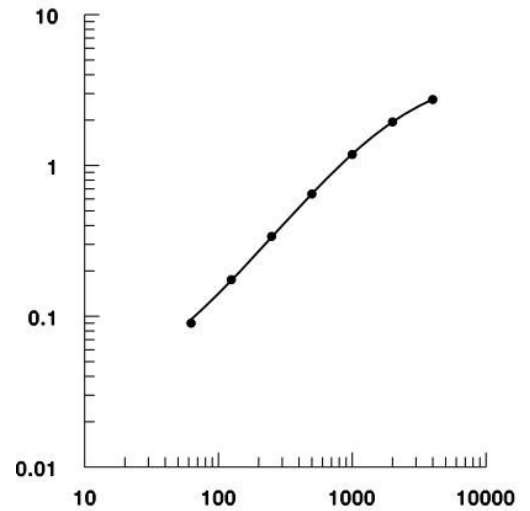
3. Configure liquid: 30 times of wash solution diluted 30 times with distilled water and reserve.
4. washing: remove Liquid, dry by swing, add washing buffer to every well, still for 30 second then remove, repeat 4 times.
5. add Biotinylated anti-IgG: Add diluted Biotinylated anti-IgG 50 $\mu$ L to all wells, Incubate for 30 min at 37 $^{\circ}$ C
6. washing: Operation with 4.
7. add streptavidin-HRP: Add streptavidin-HRP 50 $\mu$ L to all wells, Gently mix Incubate for 15 min at 37 $^{\circ}$ C
8. washing: Operation with 4.
9. color: Add Chromogen Solution A 50 $\mu$ L first, then add Chromogen Solution B 50 $\mu$ L to each well, Incubate for 15 min at 37 $^{\circ}$ C
10. Stop the reaction: Add Stop Solution 50 $\mu$ L to each well, Stop the reaction (the blue color change to yellow color immediately).
11. assay: take blank well as zero, measure the optical density (OD) at 450 nm after Adding Stop Solution and within 15 min.



## TYPICAL DATA

### CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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 the graph. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



## TROUBLE SHOOTING

*If the results are not good, please take pictures, keep the used plate and remaining reagents. Then contact our technical department. Meanwhile, you could refer to the following materials.*

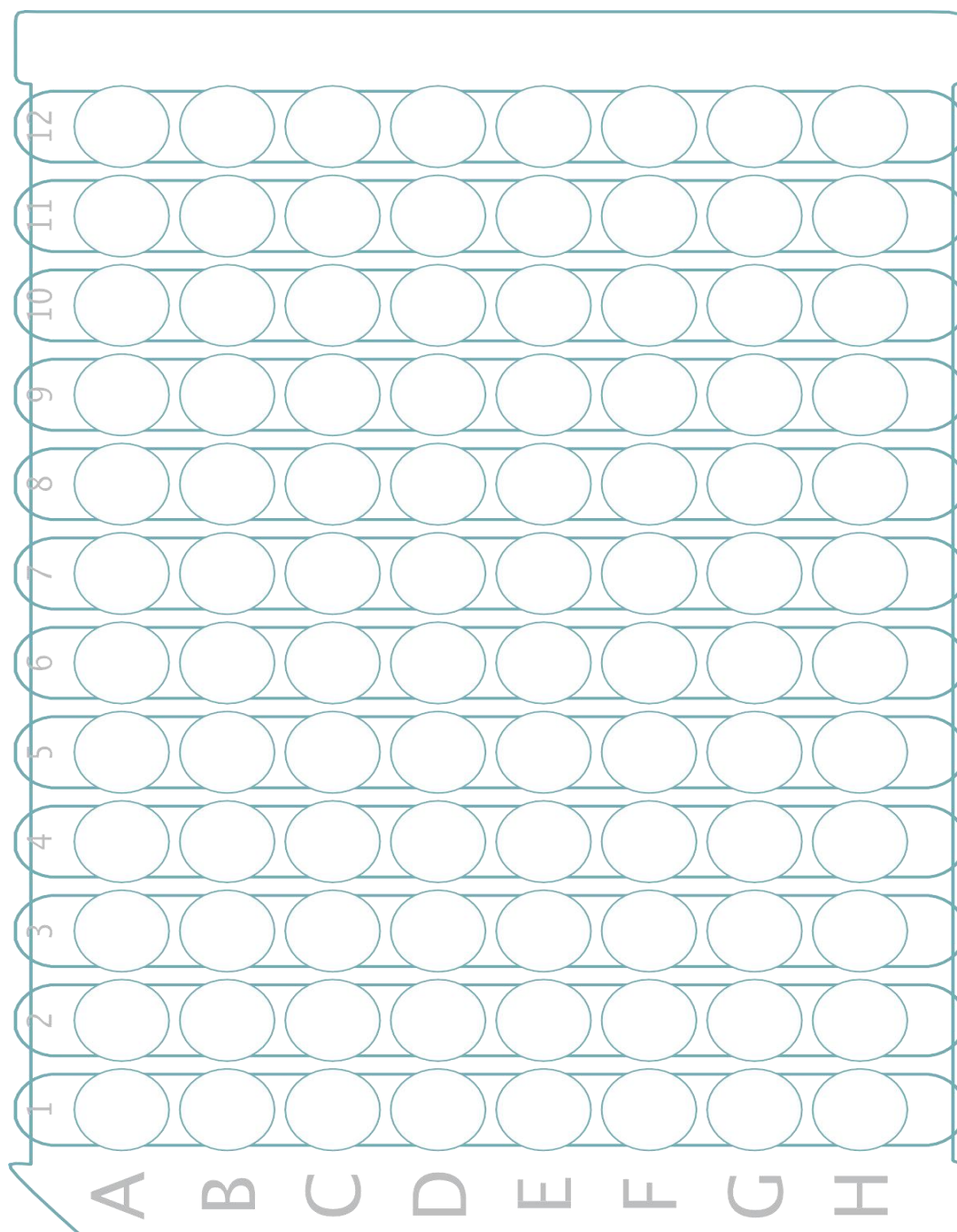
<b>Problem</b>	<b>Possible Source</b>	<b>Correction Action</b>
Poor Standard Curve	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or Chromogen Solution reagent failure	Mix conjugate & substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay

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## PLATE LAYOUT



Use this plate layout to record standards and samples assayed.

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