General DA ELISA Kit

Cat: AMS.ELK7879

96 Tests

For research use only. Not for use in diagnostic procedures.

KIT components & storage

Reagents	Quantity	Storage Condition
Pre-coated Microplate	8×12wells	4°C
Standard (lyophilized)	2	4°C
Standard Diluent Buffer	20 mL	4°C
Biotinylated-Conjugate (100x)	60 µL	4°C(lucifuge)
Biotinylated Conjugate Diluent	10 mL	4°C
Streptavidin-HRP(100×)	120 µL	4°C
HRP Diluent	12 mL	4°C
Wash Buffer (25×)	20 mL	4°C
TMB Substrate Solution	9 mL	4°C(lucifuge)
Stop reagent	6 mL	4°C
Plate Covers	4	4°C
Instruction manual	1	4°C

Special Explanation

1. Store kit at 4°C immediately upon receipt.
2. Do not use the kit after the expiration date.
3. Please check whether all components are complete after opening the package.

All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Materials Required, Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Distilled or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2μL to 1mL volumes.

Safety notes

1. This kit is sold for lab research and development use only and not for use in humans or animals.
2. Reagents should be treated as hazardous substances and should be handled with care and disposed of properly.
3. Gloves, lab coat, and protective eyewear should always be worn, Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Dopamine(DA) protein. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Dopamine(DA). Next,Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Dopamine(DA) in the samples is then determined by comparing the OD of the samples to the standard curve.

Sample collection and storage

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use .Avoid repeated freeze/thaw cycles

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use .Avoid repeated freeze/thaw cycles.

Tissue homogenates -The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v=1:9, e.g. 900 μL lysis buffer is added in 100mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10000×g. Collection the supernatant and assay immediately or aliquot and store at ≤-20℃.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Cells were then resuspended in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1500×g for 10 minutes at 2-8℃ to remove cellular debris. Assay immediately or aliquot and store at ≤-20℃.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use.

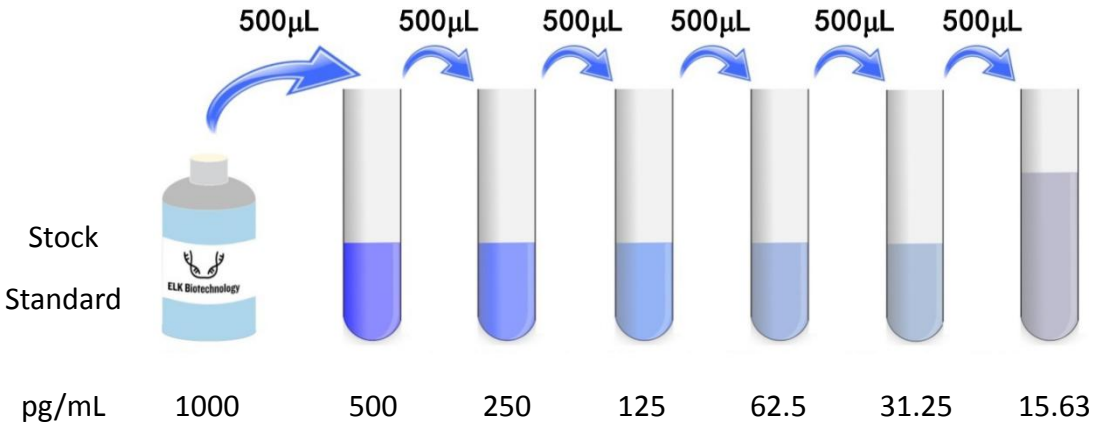
Avoid repeated freeze/thaw cycles.

Note

1. 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 repeated freeze/thaw cycles.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. If the concentration of the test material in your sample is higher than that of the standard product, please make the appropriate multiple dilution according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.

Reagent preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25x wash buffer into 1x working concentration with double steaming water.
4. Biotinylated-Conjugate (1x) - Centrifuge the vial before opening. Biotinylated-Conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10µL of Biotinylated-Conjugate with 990 µL of Biotinylated-Conjugate Diluent.
5. **Standard** -Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 1000 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.63 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



6. Streptavidin-HRP (1x) - Centrifuge the vial before opening. Streptavidin-HRP requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Streptavidin-HRP with 990 µL of HRP Diluent.
7. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note

1. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
4. If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.
5. Prepare standards within 15 minutes before assay. This standard can only be used once.
6. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells. Dispense the TMB solution within 15 minutes following the

washing of the microtiter plate.

8. It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box.

Samples preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1. Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.
2. Prepare all reagents, working standards, and samples as directed in the previous sections.
3. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2 - 8°C.
4. Set a Blank well without any solution. Add 50 µL of Standard or Sample to per well. Add 50 µL of Biotinylated -Conjugate(1x) to each well. Mix well, Cover with the adhesive films provided. Incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Streptavidin-HRP (1x) to each well. Cover with the adhesive films provided. Incubate for 30 minutes at 37°C.
7. Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each

well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

8. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.
9. Add 50 µL of Stop Solution to each well. When the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. 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 540 nm or 570 nm. Subtract readings at 540 nm or 570 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 be higher and less accurate.
11. ***Samples may require dilution. See Sample Preparation section.**

Calculation of Results

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between DA concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of DA concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

Sensitivity: 4.71 pg/mL

Detection range: 15.63-1000 pg/mL

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.

Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
Low single	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	The samples storage too long	Use new sample and repeat assay
	Incorrect incubation temperature	Ensure the incubator temperature is always 37°C
Poor Precision	Inaccurate pipetting	Check and calibrate pipettes
	Incomplete washing of wells	Ensure sufficient washing
	Contaminated wash buffer	Prepare fresh wash buffer
Poor repeatability	Time of addition is not consistent	Ensure that the sample time is consistent every time
	The washing conditions are not consistent	Ensure the frequency and strength of each wash are consistent