

Monkey Cotinine ELISA Kit

Cat: OPK0704

For research use only. Not intended for diagnostic use.

Sensitivity: Minimum detected dose is less than 0.1 ng/mL

Detection Range: 5 ng/mL – 80 ng/mL.

Specificity: This kit recognizes natural and recombinant Monkey Cotinine without crossing with structural analogs.

Kit Components & Storage

Store the kit at 2-8°C, valid for 6 months

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-coated enzyme plate	48T	96T	2-8°C, 14 days
Standard	0.3 mL × 6 tube	0.3 mL × 6 tube	2-8°C, 14 days
Sample dilution	3 mL	6 mL	2-8°C, 180 days
Biotinylated antigens	3 mL	6 mL	2-8°C, 14 days
HRP labeled avidin	3 mL	6 mL	2-8°C, 180 days
Colour-producing substrate A	3 mL	6 mL	2-8°C, 180 days
Colour-producing substrate B	3 mL	6 mL	2-8°C, 180 days
Termination fluid	3 mL	6 mL	2-8°C, 180 days
20× washing solution	15 mL	25 mL	2-8°C, 180 days
Plate cover	2 pieces	2 pieces	2-8°C

Special Explanation

1. Before use, please check whether the label and quantity of the reagent in the kit are consistent with the table.
2. If the components of the kit need to be used again, please make sure that they are not contaminated after the last use.
3. The enzyme label plate has not been used for a single time. Remember to seal it and store it at 2-8°C.

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. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2 μ L to 1 mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with reagents. In case of contact, wash thoroughly with water.

Test Principle

The kit adopts enzyme-linked immunoassay method. The purified anti- antibody is coated with microplate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with the biotin-labeled and non-labeled antigen (calibrator or specimen). The binding amount of the antibody to the biotin-labeled is inhibited by the amount of non-labeled antigen. If the amount of non-labeled antigen is large, the antibody and the biotin-labeled will bind less, and vice versa. After the reaction is equilibrium, a solid-phase antibody-biotinated will be formed, and then the enzyme- labeled avidin is added to form the solid-phase antibody-biotinated -enzyme-labeled - enzyme-abilibrated complex. After adding substrate to develop colour, the absorbance (OD value) was measured at a wavelength of 450nm using a microplate reader. As the concentration increases, the OD value gradually decreases and shows a good linear relationship. This kit has the characteristics of high sensitivity, strong specificity, good repeatability, simple operation and fast operation, and has reliable detection performance for the reduction or increase of in the serum.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

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

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

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince 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) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).

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3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
 4. Then, centrifuge the homogenates for 5 minutes at $10000 \times g$ and collect the supernatant and assay immediately or store in aliquots at $\leq -20^{\circ}\text{C}$.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein

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

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at $1000 \times g$ for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at $1500 \times g$ for 10 minutes at $2-8^{\circ}\text{C}$ to remove cellular debris. Assay immediately or store in aliquots at $\leq -20^{\circ}\text{C}$.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at $1000 \times g$ at $2-8^{\circ}\text{C}$ for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 μL lysis buffer is added in 100 mg feces), sonicated (or mashed) and centrifuged at $5000 \times g$ for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at $1000 \times g$ for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Notes

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 it 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 dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio).

Reagent Preparation

1. Before use, all components must be re-warmed for at least 60 minutes to ensure full re- warming to room temperature.
2. Concentrated washing liquid: The concentrated washing liquid taken out of the refrigerator will produce crystallization, which is a normal phenomenon. The crystallization is completely dissolved by heating in a water bath. The concentrated washing liquid and distilled water are diluted at 1:20, that is, 1 part of the concentrated washing liquid, and 19 parts of the distilled water are added.

Notes

1. It is for scientific research only and shall not be used for clinical diagnosis.
2. Used within the validity period marked by the kit, expired products shall not be used.
3. Do not mix with other manufacturers' kits or components, use the sample diluent that is equipped with the kit.
4. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately before re-measurement.
5. The heterophilic antibodies such as human anti-mouse in the sample to be tested will interfere with the detection results. Please excrete this factor before testing.
6. The test results obtained by other methods are not directly comparable to the test results of this kit.

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7. Please wear experimental clothes and latex gloves to protect yourself during the test. Especially when testing blood or other body fluid samples, please follow the National Biological Laboratory Safety Protection Regulations.
 8. Incubate strictly in accordance with the prescribed time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate immediately after use.
 9. Incorrect washing of the board can lead to inaccurate results. Make sure to suck the liquid in the pores as much as possible before adding the substrate. Do not let the micropores dry out during incubation.
 10. Eliminate residual liquid and finger prints on the bottom of the plate, otherwise it will affect the OD value.
 11. The substrate colour-producing liquid should be colourless or very light.
 12. Avoid cross-contamination of reagents and specimens to avoid causing erroneous results.
 13. Avoid direct exposure of strong light during storage and incubation.
 14. The microplate reader used for detection needs to be installed with a filter that can detect wavelengths of $450 \pm 10\text{nm}$, with an optical density range between 0-3.5. It is recommended to warm up 15 minutes in advance when using it.
 15. The EP tube and suction head used in the test are both used once and are strictly prohibited from being mixed.

Assay Procedure

All reagents and components are first restored to room temperature, and are recommended to make compound holes.

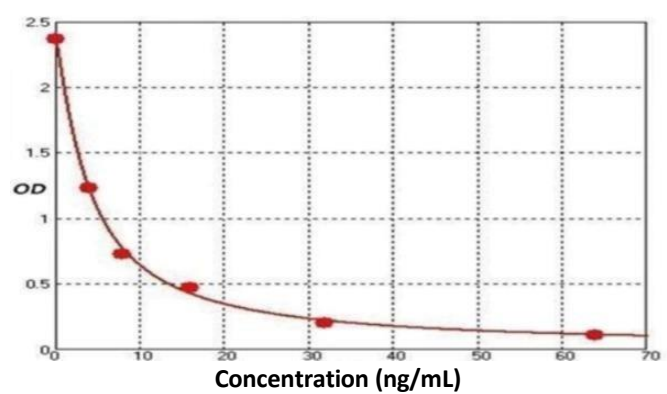
1. Prepare the working liquid of various components of the kit according to the method described in the previous instructions.
2. Remove the required slats from the aluminum foil bag, and seal the remaining slats with a self-sealing bag and put them back into the refrigerator.
3. Remove the pre-coated plate from the sealed bag and set a blank control hole without adding any liquid; set 2 wells for each calibration product, and add 50 μL of the corresponding calibration product per well; add 50 μL of the serum or quality control product to be tested for each test hole directly.
4. Add 50 μL of biotinylated antigen to all wells except the blank wells, mix well, paste the sealing membrane, and incubate at 37°C for 60 minutes.

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5. Manual washing plate: Discard the liquid in the holes, fill the wash liquid with each hole, let it stand for 10 seconds and spin dry, repeat 3 times before patting dry. Wash the plate machine. Plate: Select wash 3 times and then pat dry.
(Tip: To obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please perform the next step immediately and do not let the microplate dry.)
 6. Add 50 μL of enzyme avidin per well (except for blank control wells), mix well, paste the sealing membrane, and incubate at 37°C for 30 minutes.
 7. Manual washing plate: Discard the liquid in the holes, fill the wash liquid with each hole, let it stand for 10 seconds and spin dry, repeat 3 times before patting dry. Wash the plate machine. Plate: Select wash 3 times and then pat dry.
 8. Add 50 μL of colour developer A and 50 μL of colour developer B in each well. After oscillation and mixing, set it at 37°C to avoid light and develop colour for 15 minutes, and add 50 μL of stop solution per well.
 9. Use a microplate reader to read the wavelength of 450nm, first use a blank control hole to adjust the zero point, and then determine the optical density value (OD value) of each hole.

Calculation of Results

1. Use the concentration of the standard substance on the X-axis and the corresponding absorbance (OD value) on the Y-axis. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. (Calculate using ELISA Calc software. It is recommended to use four-parameter fitting for the standard curve, but it is not the only fitting method)
2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to determine the final value of the sample concentration.

Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only.



Note: this graph is for reference only

Precision

In-batch variation coefficient CV% is less than 10%; interbatch variation coefficient CV% is less than 15%.

Recovery

The recovery rate is between 85% and 115%.

Fast Questions and Answers

Problem description	Possible reasons	Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or addition	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each hole
Very weak or colourless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspiration and addition process to ensure that all reagents are added in sufficient order and in sufficient quantities.
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid colour development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter settings on the microplate reader
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the detection antibody is too high	Use the recommended dilution factor
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked; whether the washing solution provided in the kit is used
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to instructions
	Not terminated before reading	Stop solution should be added to each well before OD reading

Statement

1. Due to the current conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.
2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.
3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. The user should fully consider the possible usage of the sample and reserve sufficient samples before use.
4. In order to achieve good experimental results, please only use the reagents provided in our company kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may result. Please read the instructions carefully and adjust the instrument before the experiment.
6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.
7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.
8. This kit has not been compared with similar kits from other manufacturers or products that detect the same target substance using different methods, so inconsistent test results cannot be ruled out.

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9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.

For further details, contact us at

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