MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Human Cotinine ELISA Kit (Quantitative)

Product Number: ELK02J1226

Shipping and Storage

Store at 2-8℃, away from light and moisture. Validity period: 6 months.

Component

Component	96-Well Configuration	48-Well Configuration
Microplate	8 wells×12 strips	8 wells×6 strips
Standards	0.3mL×6 pipes	0.3mL×6 pipes
Sample diluent	6mL	3mL
Detection Antibody-HRP	6mL	3mL
20×washing buffer	25mL	15mL
Substrate A	6mL	3mL
Substrate B	6mL	3mL
Stop solution	6mL	3mL
Plate Sealer	2 sheets	2 sheets

Note: The concentrations of standard samples (S0-S5) are 0、3.125、6.25、12.5、25、50ng/mL, respectively.

Description

The reagent kit adopts competitive enzyme-linked immunosorbent assay (ELISA). Add the sample, standard, and HRP labeled competitive antigen to the pre coated micropores with cotinine antibody, incubate and thoroughly wash. Using substrate TMB for color development, TMB is converted to blue under the catalysis of peroxidase and to the final yellow under the action of acid. The depth of color is negatively correlated with cotinine in the sample. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader and calculate the sample concentration.

Specimen requirements

1. Serum: Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃.Avoid repeated freeze-thaw cycles.
2. Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8℃ within 30 minutes of collection. Store samples at -20℃or -80℃. Avoid repeated freeze-thaw cycles.
3. Urine, pleural and ascitic fluid, cerebrospinal fluid, and saliva: Collect using sterile tubes, centrifuge at 2-8°C for 20 minutes (3000 rpm). Carefully collect the supernatant or store at -20°C or -80°C for future use. However, repeated freeze-thaw cycles should be avoided.
4. Cell culture supernatant: Collect using sterile tubes, centrifuge at 2-8°C for 20 minutes (3000 rpm), carefully collect the supernatant, or store at -20°C or -80°C for later use. However, repeated freeze-thaw cycles should be avoided.
5. Animal tissue samples: Wash the tissue with pre cooled PBS (0.01M, pH=7.4) to remove residual blood, weigh and cut the tissue into small pieces. Mix the shredded tissue with the corresponding volume of PBS (usually in a weight to volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to experimental needs, and records should be kept. Recommend adding protease inhibitors to PBS and grinding thoroughly on ice (at low temperature) in a glass homogenizer. Alternatively, grinding can be performed in a tissue grinder. If further lysis of tissue cells is required, the homogenate can be sonicated and finally centrifuged at 5000×g for 10 minutes at 2-8℃. The supernatant can be collected for testing. For other tissue samples that are not thoroughly homogenized in a glass homogenizer

or tissue grinder, they should be thoroughly ground in liquid nitrogen.

6. Plant tissue samples: Wash the tissue with pre cooled PBS (0.01M, pH=7.4) to remove residual soil, pesticides, etc. After weighing, cut the tissue into small pieces. Mix the shredded tissue with the corresponding volume of PBS (usually in a weight to volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to experimental needs, and records should be kept. Recommend adding protease inhibitors to PBS and grinding thoroughly on ice (at low temperature) in a glass homogenizer. Alternatively, grinding can be performed in a tissue grinder. If further lysis of tissue cells is required, the homogenate can be sonicated and finally centrifuged at 5000×g for 10 minutes at 2-8℃. The supernatant can be collected for testing. The supernatant should be divided into single doses and stored at -20℃ or -80℃, avoiding repeated freezing and thawing.
7. Cell sample: Animal cells: For adherent cells, gently wash the cells with an appropriate amount of pre cooled PBS and separate them by trypsin digestion. Collect cells by centrifugation at 1000 ×g for 5 minutes (suspended cells can be directly collected by centrifugation). Discard the supernatant and wash the cells three times with cold PBS. Resuspend cells in cold PBS at a concentration of 1 × 107 cells/mL. Use an ultrasonic disruptor to thoroughly break down cells, causing them to break down and release intracellular components. Centrifuge at 2-8℃ for 20 minutes (3000 rpm), then carefully collect the supernatant for testing; Plant cells: Dilute the cell suspension with PBS at pH 7.2-7.4 to achieve a cell concentration of around 1 million/ml, place it on an ice box, and use an ultrasonic disruptor to thoroughly crush the cells. Centrifuge at 2-8℃ for 20 minutes (3000 rpm), carefully collect the supernatant for testing.
8. Throat swab: Add 2mL of PBS with pH 7.2-7.4, dissolve the head of the throat swab, shake well, use tweezers to remove the throat swab and squeeze the liquid dry. Centrifuge at 2-8℃ for about 20 minutes (2000-3000 rpm), and carefully collect the supernatant. Pack one portion for testing, freeze the rest for later use. If sediment forms during storage, centrifuge again before loading for testing.

Extract specimens as soon as possible after collection, and conduct experiments as soon as possible after extraction. If the experiment cannot be conducted immediately, the sample should be used within 6 days when it can be stored at 2-8℃. Otherwise, it must be stored at -20℃ (≤ 1 month) or -80℃ (≤ 2 months), and repeated freezing and thawing should be avoided. If precipitation forms during storage, it should be centrifuged again before sample testing. Samples containing NaN3 cannot be detected because NaN_3 inhibits the activity of horseradish peroxidase (HRP).

Materials required but not supplied

1. ELISA reader (450nm)
2. High precision sampler and nozzle: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃ constant temperature box

Reagent Preparation

20×dilution of washing buffer: Dilute distilled water at a ratio of 1:20, which means adding 19 parts of distilled water to 1 part of 20×washing buffer.

Washing method

1. Manual board washing: Shake off the liquid in the holes, fill each hole with washing solution, let it stand for 1 minute, shake off the liquid in the holes, pat dry on absorbent paper, and wash the board 5 times in this way.
2. Automatic washing machine: Inject 350μL of washing solution into each well, soak for 1 minute, and wash the plate 5 times.

Protocol

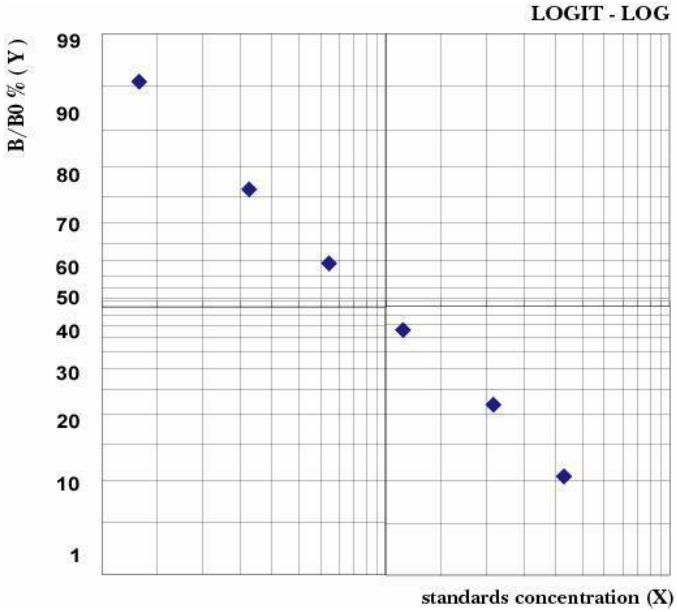
1. Take out the required plate strips from the aluminum foil bag after equilibrating at room temperature for 20 min. Seal the remaining strips with a self-sealing bag and store back at 4°C.
2. Set standard wells and sample wells. Add 50μL of standards with different activities to each standard well.
3. Add 25μL of the test sample to the sample well first, and then add 25μL of sample diluent.

4. Add 50μL of horseradish peroxidase (HRP) labeled competitive antigen to each well of the standard and sample wells, seal the reaction well with a sealing plate membrane, and incubate at 37 ℃ in a water bath or constant temperature incubator for 60 minutes.
5. Discard the liquid, pat dry on absorbent paper, fill each well with detergent, let it stand for 1 minute, shake off the detergent, pat dry on absorbent paper, repeat washing the board 5 times (or use a board washing machine).
6. Add 50μL of substrate A and B to each well, and incubate at 37℃ in the dark for 15 minutes.
7. Add 50μL of termination solution to each well and measure the OD value of each well at a wavelength of 450nm within 15 minutes.

Result judgment

1. Read the OD values of each well on an enzyme-linked immunosorbent assay reader with a wavelength of 450nm within 15 minutes;
2. Calculation of percentage binding rate: Assuming S0 tube counts as B0 and each standard tube or sample tube counts as B, the formula for calculating percentage binding rate is as follows: B/B0=B/B0 × 100%
3. Logit calculation: The formula for calculating the logit value of each standard point or sample tube is as follows: logit=ln (B/B0)/(1-B/B0)
4. Divide the mean OD of the standard sample by the OD of point 0 of the standard sample to obtain the percentage binding rate of the standard point, and plot it on log log log coordinate paper.
5. Log log double logarithmic standard curve: The horizontal axis on the coordinate paper from left to right, with the first 1-9 representing the first decimal and the second 1-9 representing the second decimal. The third 1-9 is represented as the third decimal. The vertical axis of the coordinate paper represents the percentage (1-99), which is the percentage combination rate of each standard absorbance value. Take a straight line passing through each point. Require as many points as possible to be on the line, while the remaining points are evenly distributed on both sides of the line. The percentage binding rate of the sample is also calculated from the absorbance value, and then the point on the straight line is found from the corresponding binding rate on the vertical axis. The concentration on the horizontal axis corresponding to this point is the concentration of the sample, which does not need to be converted.
6. Manual processing: Take the log value of the standard concentration as the horizontal axis and the corresponding logit value as the vertical axis. Draw a standard curve (ideally a straight line) on a regular coordinate paper or on a logit log coordinate paper with the standard concentration as the horizontal axis and the corresponding B/B0 as the vertical axis. The concentration value of the sample can be determined from the coordinate paper based on its B/B0. If using regular coordinate paper, the final concentration value should be the inverse number obtained.
7. Automatic processing: Using logit log or four parameter data processing mode, the results are automatically calculated by the computer.
8. Sensitivity: 0.1ng/mL

Note: This calibration chart is for reference only. Please refer to the final calibration chart for actual testing.



Test kit performance

1. Accuracy: The correlation coefficient R value between the linear regression of the standard and the expected activity is ≥ 0.9900.
2. Sensitivity: The minimum detectable activity is less than 0.1ng/mL.
3. Detection range: 3.125-50ng/mL.
4. Specificity: No cross-reaction with other soluble structural analogs.
5. Repeatability: Both intra-plate and inter-plate coefficients of variation are less than 15%.

Note

1. The reagent kit should be stored at 2-8℃ and equilibrated at room temperature for 20 minutes before use. The concentrated washing solution taken out of the refrigerator will have crystals, which is a normal phenomenon. Heating in a water bath will completely dissolve the crystals before use.
2. The Flat noodles not used in the experiment should be immediately put back into the self sealing bag, sealed (low-temperature drying) and stored.
3. The S0 standard with a concentration of 0 can be considered as a negative control or blank; When operating according to the instructions, the sample has already been diluted 5 times, and the actual concentration of the sample is obtained by multiplying the final result by 5.
4. Strictly follow the time, liquid dosage, and sequence indicated in the instructions for incubation operation.
5. Shake all liquid components thoroughly before use.
6. Take down the enzyme labeled Flat noodles that cannot be used temporarily and put them into aluminum foil bags for standby, and store them at 2-8℃. Suggest using it as soon as possible.

Disclaimers

1. The reagent kit is for research purposes only and should not be used for clinical experiments or mouse experiments. Any consequences arising therefrom shall be borne by the experimenter, and our company shall not be held responsible.
2. Strictly follow the instructions for operation. If the experimenter violates the instructions, the consequences shall be borne by the experimenter.