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Mouse/Rat Cotinine ELISA

Catalog Number: CO096D-100 (96 Tests)

INTENDED USE

The Calbiotech Cotinine Direct ELISA Kit is intended for the measurement of Cotinine in Mouse/Rat serum or urine. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Cotinine is a primary metabolite of nicotine and is widely used as a biomarker of tobacco exposure. This assay is intended for research in rodent models to study nicotine metabolism, pharmacokinetics, and the biological impact of environmental tobacco smoke.

PRINCIPLES OF THE TEST

The Calbiotech Cotinine kit is a solid phase competitive ELISA. The samples and Cotinine enzyme conjugate are added to the wells coated with anti-Cotinine antibody. Cotinine in the samples competes with a Cotinine enzyme (HRP) conjugate for binding sites. Unbound Cotinine and Cotinine enzyme conjugate is washed off by washing step. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cotinine in the samples. A standard curve is prepared relating color intensity to the concentration of the Cotinine.

MATERIALS PROVIDED	96 TESTS
1. Microwell coated with polyclonal Ab to Cotinine	12x8x1
2. Standard Set (ready to use)	0.5 mL
3. Control Set (ready to use)	0.5 mL
4. Cotinine HRP Enzyme Conjugate (ready to use)	12 mL
5. TMB Substrate (ready to use)	12 mL
6. Stop Solution (ready to use)	12 mL
7. 20X Wash Concentrate: 1 bottle	25 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

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WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature

ASSAY PROCEDURE.

All reagents must be brought to room temperature (20-25°C) before use.

1. Pipette 10 µl of standards, controls and specimens into selected well in duplicate.
2. Add 100 µl of the Enzyme Conjugate to each well. Shake the plate, 10-30 seconds, to ensure proper mixing.
3. Incubate for 60 minutes at room temperature (20-25°C) preferably in the dark.
4. Wash the wells 3 times with 300 µl 1X Wash Buffer using either a suitable plate washer or wash bottle taking care not to cross contaminate wells.
5. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate, does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.
6. Add 100 µl of Substrate reagent to each well.
7. Incubate for 30 minutes at room temperature, preferably in the dark.
8. Add 100 µl of Stop Solution to each well. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450nm with in 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

1. The standard curve is constructed as follows:
2. Check Cotinine standard value on each standard vial.
3. To construct the standard curve, plot the absorbance for Cotinine standards (vertical axis) versus Cotinine standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
4. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a standard curve

	OD 450 nm	Conc. ng/mL
Std 1	2.92	0
Std 2	1.53	5
Std 3	0.85	10
Std 4	0.43	25
Std 5	0.27	50
Std 6	0.16	100