



Human Cotinine ELISA

Catalog Number: IB19137 (96 Tests)

INTENDED USE

The Cotinine ELISA Kit is intended for the measurement of Cotinine in human serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Exposure to tobacco smoke can be detected by measuring nicotine and its metabolites. Nicotine has a short half life and is not used as a marker for tobacco smoke exposure. Cotinine due to its longer half life has been used in research as a reliable marker for smoking status and smoking cessation studies. The Cotinine Direct ELISA Kit is designed for the detection Cotinine in serum. It can also be adapted for other fluids.

PRINCIPLES OF THE TEST

The Cotinine kit is a solid phase competitive ELISA. The samples, cotinine antibody reagent, and Cotinine enzyme conjugate are added to the wells coated with Goat anti-rabbit IgG. Cotinine in the samples competes with a Cotinine enzyme (HRP) conjugate for binding sites on the antibody reagent which is then absorbed onto the plate. 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.

For Research Use Only
 Manufactured For IBL America
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MATERIALS PROVIDED	96 TESTS
Microwell coated with Goat x Rabbit IgG	12x8x1
Standard Set (ready to use)	0.25 mL
Control Set (ready to use)	0.25 mL
Cotinine Enzyme Conjugate (ready to use)	7 mL
Anti-Cotinine Antibody Reagent (ready to use)	12 mL
TMB Substrate (ready to use)	12 mL
Stop Solution (ready to use)	12 mL
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.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls 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, 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

1. This Cotinine Direct ELISA Kit is to be used with human serum. This assay has not tested for all possible applications. Cutoff criteria are important in deciding the sample dilution.
2. Specimens to which sodium azide has been added affect the assay.

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 Cotinine Antibody Reagent to each well.
3. Add 50 µL Cotinine Enzyme Conjugate to each well. Shake the plate, 10-30 seconds, to ensure proper mixing.
4. Incubate for 60 minutes at room temperature (20-25°C) preferably in the dark.
5. Briskly shake out the contents of the wells.
6. Rinse the wells 3 times with diluted Wash Solution (350 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
7. Add 100 µl of TMB Substrate into each well.
8. Incubate for 30 minutes at room temperature.
9. Stop the enzymatic reaction by adding 50 µl of Stop Solution into each well.
10. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop 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 xy scatterplot. Draw a point-to-point 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	2.24	0.0
Std 2	1.78	0.6
Std 3	1.11	1.9
Std 4	0.47	5.6
Std 5	0.17	16.7
Std 6	0.07	50.0