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Product Information

Mouse/Rat Cotinine ELISA kit

Catalog Number: EA100902
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Mouse/Rat Cotinine Direct ELISA Kit is intended for the measurement of Cotinine in mouse/rat serum and urine.

Background

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 and urine. It can also be adapted for other fluids.

Principle of the Test

The 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with polyclonal Ab to Cotinine	12x8x1
2. Standard Set (ready to use)	0.5 ml
3. Cotinine HRP Enzyme Conjugate (ready to use)	12 ml
4. TMB Substrate (ready to use)	12 ml
5. Stop Solution (ready to use)	12 ml
6. 20 x Wash concentrate: 1 bottle	25 ml

Materials and Equipment Required but 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

Disclaimer
This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation
1. This Cotinine Direct ELISA Kit is to be used with mouse/rat urine or 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 1 x Wash buffer by adding the contents of the bottle (25 ml, 20 x) to 475 ml of distilled or deionized water. Store at room temperature.

Assay Procedure
- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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 1 x 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 within 15 minutes after adding the stopping solution.

Calculations of Results
The standard curve is constructed as follows:
1. Check Cotinine standard value on each standard vial.
2. 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.

3. 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

Standards	Conc. (ng/ml)	Absorbance (450nm)
STD1	0	2.92
STD2	5	1.53
STD3	10	0.85
STD4	25	0.43
STD5	50	0.27
STD6	100	0.16

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