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# Human Cotinine ELISA Kit (Competitive EIA)

## Catalog number: EK7035

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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# Human Cotinine ELISA Kit (Competitive EIA)

**Catalog Number:** EK7035

**Size:** 96 wells/kit

**Sample Type:** Serum and Plasma

**Sensitivity:** 1 ng/ml

**Assay Range:** 5-100 ng/ml

**Storage:** Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

## Introduction

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.

## Kit Components

| Description | Quantity |
|---|---|
| Microwell coated with polyclonal Ab to Cotinine | 12x8x1 |
| Standard Set (ready to use) | 0.5 ml |
| Cotinine HRP Enzyme Conjugate (ready to use) | 12 ml |
| TMB Substrate (ready to use) | 12 ml |
| Stop Solution (ready to use) | 12 ml |
| Wash Concentrate, 20X: 1 bottle | 25 ml |

### Standard Concentrations and example data

| | OD 450 nm | Conc. ng/mL |
|---|---|---|
| Std 1 | 2.9 | 0 |
| Std 2 | 2.25 | 5 |
| Std 3 | 1.5 | 10 |
| Std 4 | 0.77 | 25 |
| Std 5 | 0.47 | 50 |
| Std 6 | 0.27 | 100 |

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## Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

## WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. 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
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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 COLLECTION AND HANDLING

1. This Cotinine Direct ELISA Kit is to be used with human 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 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 ul of standards, controls and specimens into selected well in duplicate.
2. Add 100 ul of the Enzyme Conjugate to each well. Shake the plate, 10-30 seconds, to ensure proper mixing.

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3. Incubate for 60 minutes at room temperature (20-25. C) preferably in the dark.
4. Wash the wells 3 times with 300 ul of 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 ul of Substrate reagent to each well.
7. Incubate for 30 minutes at room temperature, preferably in the dark.
8. Add 100 ul 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

The standard curve is constructed as follows:
2. Check Cotinine standard value on each standard vial.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard 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.