



# Cotinine ELISA Kit

(Catalog # K4152-100, 100 assays; Store at 4°C)

rev 11/17

## I. Introduction:

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. BioVision's Cotinine Direct ELISA Kit is designed for the detection Cotinine in serum and urine. It can also be adapted for other fluids.

## II. Application:

Detect cotinine in serum and urine by solid phase competitive ELISA. For research use only.

## III. Specificity:

Human

## IV. Sample Type:

Serum or urine

## V. Kit Contents:

Components	K4152-100	Part No.
Microwell coated with anti-Cotinine Ab	12 strips x 8 wells	K4152-100-1
Standard Set (6 tubes)	0.5 ml x 6	K4152-100-2.x
Cotinine HRP Enzyme Conjugate	12 ml	K4152-100-3
TMB Substrate	12 ml	K4152-100-4
Stop Solution	12 ml	K4152-100-5
Wash Buffer (20X)	25 ml	K4152-100-6

## VI. User Supplied Reagents and Equipment:

- Distilled or deionized water
- Adjustable Precision pipettes and disposable pipette tips
- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper

## VII. Storage Conditions:

- Kit can be used within one year if stored properly at 4°C.
- Keep microwells sealed in a dry bag with desiccants.
- Avoid expose test reagents to heat, sun or strong light.

## VIII. Sample and Reagent Preparation:

- This Direct ELISA Kit is designed to be used with human urine or serum. Cutoff criteria are important in deciding the sample dilution.
- Prepare 500 ml 1X Wash buffer by adding 475 ml of distilled water to the Wash buffer concentrate (20X)
- Including sodium azide in samples would affect the assay.

## IX. Assay Protocol:

### Precaution:

- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth.
- 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.

### Assay Procedure:

\*\* Bring all specimens and kit reagents to room temperature and gently mix 30 minutes before the assay.

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 preferably in the dark.
4. Wash the wells 6 times with 300 µl of **1X Wash buffer** using either a suitable plate washer or wash bottle. Be careful not to cross contaminate wells.
5. Invert wells and vigorously tap on dry absorbent paper to ensure all residual moisture is removed. If using an automated system, ensure that the final aspiration on the wash cycle aspirates all liquid from either side of the well.  
Note: This step is critical to ensure that residual enzyme conjugate, does not skew results.
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.

**FOR RESEARCH USE ONLY! Not to be used on humans.**



9. Read O.D. at 450 nm using ELISA reader within 15 min.

**X. Result Interpretation:**

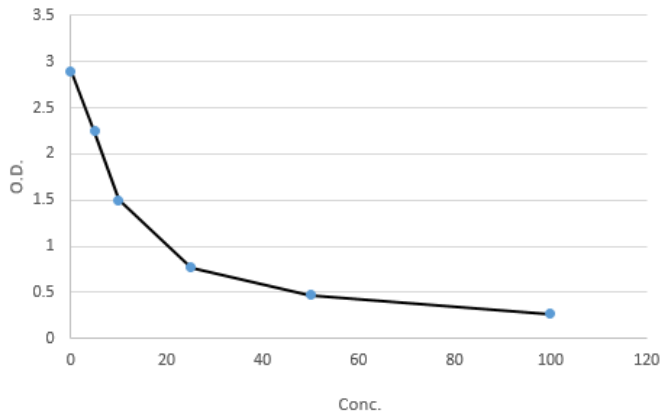
**Calculation:**

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 using curve fitting method.

**Example of a Standard Curve:**

Standard	OD (450 nm)	Part No.
Standard 1 (0 ng/ml)	2.90	K4152-100-2.1
Standard 2 (5 ng/ml)	2.25	K4152-100-2.2
Standard 3 (10 ng/ml)	1.50	K4152-100-2.3
Standard 4 (25 ng/ml)	0.77	K4152-100-2.4
Standard 5 (50 ng/ml)	0.47	K4152-100-2.5
Standard 6 (100 ng/ml)	0.27	K4152-100-2.6

Example of Standard Curve



**XI. RELATED PRODUCTS:**

- Cytochrome P450 Reductase (CPR) Human ELISA Kit (Cat. No. K7871-100)
- Aromatase (human) ELISA kit (Cat. No. K3599-100)