**Product Information**

**Cotinine ELISA**

Catalog Number **SE120039**  
Storage Temperature 2–8 °C

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**TECHNICAL BULLETIN**

**Product Description**  
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 ELISA Kit is designed for the detection of cotinine in serum and urine. It can also be adapted for other fluids.

The Cotinine ELISA 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 are 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.

The Cotinine ELISA Kit is intended for the measurement of cotinine in serum and urine.

**Components**

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with polyclonal Ab to Cotinine</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Standard Set (ready to use)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Cotinine HRP Enzyme Conjugate (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution (ready to use)</td>
<td>12 ml</td>
</tr>
</tbody>
</table>

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**Reagents and Equipment Required but Not Provided.**
- Distilled or deionized water  
- Precision pipettes  
- Disposable pipette tips  
- ELISA reader capable of reading absorbance at 450 nm  
- Absorbent paper or paper towel  
- Graph paper

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

**Sample Preparation**
1. The Cotinine 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.

**Storage/Stability**  
Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun, or strong light.
Procedure

Notes: 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 samples be run in duplicate.

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.

All reagents must be brought to room temperature (18–26 °C) before use and gently mix.

1. Pipette 10 µl of standards, controls and specimens into selected wells.
2. Add 100 µl of the Cotinine HRP Enzyme Conjugate to each well. Shake the plate 10–30 seconds to ensure proper mixing.
3. Incubate for 60 minutes at room temperature (18–26 °C) preferably in the dark.
4. Wash the wells 6 times with 300 µl of distilled water 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 TMB 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 450 nm within 15 minutes after adding the stopping solution.

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.

Example of Standard Curve

<table>
<thead>
<tr>
<th>OD 450 nm</th>
<th>Concentration ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>2.90</td>
</tr>
<tr>
<td>Std 2</td>
<td>2.25</td>
</tr>
<tr>
<td>Std 3</td>
<td>1.50</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.77</td>
</tr>
<tr>
<td>Std 5</td>
<td>0.47</td>
</tr>
<tr>
<td>Std 6</td>
<td>0.27</td>
</tr>
</tbody>
</table>

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

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