ab285286 – Cotinine ELISA Kit

For the quantitative measurement of Cotinine in serum and urine.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285286

Storage and Stability

This kit can be stored at 4°C for up to 12 months from the date of shipment. Keep microwells sealed in a dry bag with desiccants. Avoid exposure to heat, sun or strong light.

Materials Supplied

Item	Quantity	Storage Condition
Anti-Cotinine A pre-coated Microplate	8 wells ×12 wells	4°C
Standard Set (6 tubes)	6 x 0.5 mL	4°C
Cotinine HRP-Enzyme Conjugate	12 mL	4°C
TMB Substrate	12 mL	4°C
Stop Solution	12 mL	4°C
20X Wash Buffer	25 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

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.
- Sodium azide in samples would affect the assay.

1X Wash buffer: Prepare 500 mL 1X Wash buffer by adding 475 mL of distilled water to the Wash buffer concentrate (20X).

Assay Protocol

- Bring all reagents and samples to room temperature and gently mix 30 minutes prior to the assay.

1. Pipette 10 µL of standards, controls and specimens into selected wells in duplicate.
2. Add 100 µL of the Enzyme Conjugate to each well. Shake the plate for 10-30 seconds to mix thoroughly.
3. Incubate at room temperature for 60 minutes, 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.
   Δ 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 at room temperature for 30 minutes, preferably in the dark.
8. Add 100 µL of Stop Solution to each well. Shake the plate gently to mix the solution.
9. Read O.D. at 450 nm using a microplate reader within 15 minutes.

Calculation

1. Check Cotinine standard value on each standard vial.
2. To construct the standard curve, plot the absorbance for Cotinine standards (Y-axis) vs. Cotinine standard concentrations (X-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.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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