DRG® Cotinine (Urine) ELISA (EIA-1377 and EIA-1726)

IVD

REVISED 17 MAR. 2005 (VERS. 1.1)

1 INTENDED USE

The Cotinine (Urine) ELISA is intended for use in the qualitative and semi-quantitative determination of Cotinine in Urine.
Other kits are available for serum, plasma and saliva.

2 REAGENTS

1. **Anti-Cotinine Coated Plate** - (1 plate) , [5 plates]
   Anti-Cotinine antibody immobilized on a polystyrene plate. 12 x 8 wells in break-a-part format.
   Store at 2-8°C.
2. **Cotinine Enzyme Conjugate** -- (15 mL) , [60 mL]
   Buffered protein reagent with stabilizers. Ready to use. Store at 2-8°C.
3. **Wash Buffer Concentrate (30 x)** - (50 mL) , [66 mL]
   Requires dilution with distilled water before use.
   Dilute contents of the vial to 1500mL with distilled water.
4. **Substrate Solution** - (20 mL) , [60 mL]
   One bottle containing 3,3',5,5'- tetramethylbenzidine.
5. **Stop Solution** - (20 mL) , [60 mL]
   1 Molar Sulphuric acid. Treat as corrosive.
6. **Negative Calibrator** -- (1 mL) , [4 mL]
   Urine matrix negative for Cotinine
7. **Positive Calibrators** – (1 mL each level) , [4 mL, each level]
   - Urine matrix containing 50 ng/mL Cotinine
   - Urine matrix containing 500 ng/mL Cotinine
   - Urine matrix containing 5000 ng/mL Cotinine

3 WARNING AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is not recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Solution.
   Discard Substrate Solution if obvious blue colour develops.
3. Do NOT mouth pipette reagents. Handle all specimens and reagents as if potentially infectious.
4. Keep all containers closed when not in use to avoid microbial contamination.
5. Do NOT use reagents after the expiration date.
6. Do NOT mix reagents from different kits or manufacturers.
7. Do NOT freeze reagents.
8. It is suggested that all reagents be kept out of direct sunlight wherever possible.
9. Stop Solution is corrosive; handle with care.
10. Sample addition should take no longer than 30 minutes.

4 STORAGE/STABILITY

Store all reagents at 2-8°C.
The stability of the Cotinine Microplate EIA kit is a minimum of 6 months from the date of manufacture when stored at 2-8°C. The expiration date appears on all components.

5 ASSAY PROCEDURE

Prepare wash buffer by 1:30 dilution with distilled water

1. Note: Allow all reagents to come to room temperature (20-27°C) before use. At the discretion of the operator, all samples, calibrators, and controls may be tested in duplicate.
2. Add 10 µL of sample, calibrator, or control to each well. (See note 10 above)
3. Add 100 µL of Cotinine Enzyme into each well.
4. Incubate for 30 minutes at room temperature.
5. Wash the plate four times with 350 µL of Wash Buffer.
6. Add 100 µL of Substrate Solution to each well and incubate for 30 minutes at room temperature.
7. Add 100 µL of Stop Solution to each well.
8. Measure the absorbance at 450 nm within 30 minutes.

6 QUALITY CONTROL

For qualitative assays, the Negative Control must have an absorbance greater than the Cut-off Calibrator.
The Positive Control must have an absorbance less than the Cut-off Calibrator.

7 LIMITATIONS OF THE PROCEDURE

If there is any possibility that the specimen is adulterated, a new specimen should be collected.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.

8 PERFORMANCE

Specificity
The following compounds were tested for cross-reactivity at 10,000 ng/mL. None were found to cross-react:

Acetylsalicylic Acid	**Niacinamide**
Amitriptyline	Nicotine
Amobarbital	Nicotinic Acid
DL Amphetamine	Norethindrone
Ascorbic Acid	Penicillin-G
Caffeine	Phenylbutazone
Cocaine	Phenylpropanolamine
Dextromethorphan	Quinine
Dimethylaminoantipyrine	Zomepirac
Methoxymethyl-naphthalene-acetic acid	

The following compounds were tested for cross-reactivity at 10,000 ng/mL.

Compound	% Cross Reactivity
Cotinine	100
3-Hydroxycotinine	10
Nicotine	0

9 INTERPRETATION

9.1 Qualitative Result

Positive result:
Any sample with an absorbance less than or equal to the chosen Cut-off Calibrator is considered a positive.

Negative result:
Any sample with an absorbance greater than the chosen Cut-off Calibrator is considered a negative.

9.2 Semi-Quantitative Result

Where estimates of relative total drug concentrations are desired, a Calibration curve should be prepared with four or more calibration points to allow comparison of absorbance values of the unknowns.
Intermediate calibrators can be prepared by suitable dilution.