COTININE Direct Elisa

Catalog Number: BQ086D (96 wells)

EXPLANATION OF THE TEST

This Cotinine Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of cotinine in serum and urine. 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.

PRINCIPLES OF THE PROCEDURE

This Cotinine Direct ELISA Kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture.

A 10 µl. aliquot of a diluted unknown specimen is incubated with a 100 µl. dilution of enzyme (Horseradish peroxidase) labeled Cotinine derivative in micro-plate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 1 ng/ml

REAGENTS

COTININE Direct ELISA Kit Contents

Component	96 test Kit
96 well Micro-plate	1
Cotinine Conj.	15 mL
Neg Serum Std	1 mL
Cotinine 5 ng/mL	1 mL
Cotinine 10 ng/mL	1 mL
Cotinine 25 ng/mL	1 mL
Cotinine 50 ng/mL	1 mL
Cotinine 100 ng/mL	1 mL
Neg Urine Std	2 mL
Cotinine Pos Std	2 mL
TMB Substrate	30 ml
Stop Reagent	25 ml

96 well micro-plate. The micro-plate is coated with polyclonal antibody to Cotinine via a spacer chain to provide optimally oriented binding sites. The plates are sealed in a moisture and air barrier pouch with a dessicant.

Cotinine-Enzyme Conjugate The conjugate solution contains a Cotinine derivative labeled with horseradish peroxidase in a buffered, protein solution with stabilizers, pH 7.6 containing non azide preservatives.

Negative Serum Standard. This bottle contains drug free rabbit serum containing azide free preservatives.

Serum Cotinine Standards. These bottles contain 5, 10, 25, 50 and 100 ng/mL of Cotinine dissolved in a rabbit serum non azide preservative.

Negative Urine Standard. This bottle contains drug free synthetic urine matrix containing azide free preservatives Urine Cotinine Positive Standard. This bottle contains 500 ng/mL of cotinine in a synthetic urine matrix containing azide free preservatives.

TMB chromogenic substrate. The color reagent contains 3,3',5,5' tetramethylbenzidine and urea peroxidase in buffer.

Stop Reagent This contains 1 N hydrochloric acid.

Materials and Equipment

Materials and equipment required but not supplied with this Cotinine Direct ELISA Kit are itemized below

12x75 mm Disposable Glass or Plastic Culture Tubes to predilute samples (if required).

Manual or electronic micropipets (single channel or multichannel) or automated pipetting stations.

Refrigerator (for kit storage).

Interval Timer.

Wash bottle or Plate Washer.

Microplate reader capable of reading at 450 nm. And 650 nm.

Precautions

1. Not for Internal or External Use in Humans or Animals.
2. There should be no eating or drinking within work area.
3. Always wear gloves and a protective lab coat.
4. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
5. **Do not add sodium azide to samples as preservative.**
6. **Do not use external controls containing sodium azide.**
7. Use disposable pipet tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
8. Do not pour chromogenic substrate back into container after use.
9. Do not freeze reagents.
10. Do not mix reagents from different kit lot numbers.
11. Keep reagents out of direct sunlight.
12. Handle stop reagent with care, since it is corrosive.
13. Bring all reagents to room temperature.
14. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pippetting.
15. Ensure the bag containing the micro-plate strips and dessicant is well sealed if only a few strips are used.

General. Precise pipetting is the essence of successful immunoassay. It is critical to pipet right at the center and bottom of each well to ensure good replicates and coefficents of variation Micropipets supplied by "Eppendorf" or "SMI" with disposable tips are excellent when used carefully according to instructions to insure the necessary accuracy. New automatic dispensers improve reliable delivery.

Storage. The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2 – 4 °C.

Indications of Deterioration. A drop of greater than 50% in the A0 (zero-standard absorbance reading) for a constant incubation time indicates deterioration of the antibody plate, enzyme conjugate or chromogenic substrate. A significant shift of the standard curve to the right would result from deterioration of the standards. Development of blue color in the chromogenic substrate without the addition of enzyme conjugate indicates contamination of the substrate.

SPECIMEN COLLECTION

Precautions.

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.

Additives.

Specimens to which sodium azide has been added affect the assay.

DETAILS OF THE PROCEDURE.

All reagents must be brought to room temperature (20-25 °C) before use.

The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipettor. Use urine calibrators for urine and serum calibrators for serum. Depending on the cutoffs a sample dilution may be required for urine applications.

1. Add 10 µl. of calibrators and standards to each well in duplicate.
2. Add 10 µl. of the specimens in duplicate (recommended) to each well.
3. Add 100 µl. of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
4. Incubate for 60 minutes at room temperature (20-25 °C) preferably in the dark, after addition of enzyme conjugate to the last well.
5. Wash the wells 6 times with 350 µl. distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells.
6. 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.
7. Add 100 µl. of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
8. Incubate for 30 minutes at room temperature, preferably in the dark.
9. Add 100 µl. of Stop Solution to each well, to change the blue color to yellow.
10. Measure the absorbance at a dual wavelength of 450 nm and 650 nm.
11. Wells should be read within 1 hour of yellow color development.
The following data represent a typical dose/response serum cotinine curve.

Cotinine ng/ml	Absorbance
0	1.759
5	1.075
10	0.865
25	0.691
50	0.495
100	0.419

The dose/response curve shown above should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or a cutoff calibrator should be run with every plate.

RESULTS
If the average sample absorbance is equal to or less than the average absorbance of the laboratory positive reference standard the sample is POSITIVE for Cotinine. If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called NEGATIVE for Cotinine.

Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve.

SPECIFIC PERFORMANCE CHARACTERISTICS
Accuracy.
20 urine samples from non smokers were screened with This Cotinine ELISA method. All 20 samples screened negative with the ELISA method. 15 samples from smokers which contained various amounts of Cotinine were screened with This Cotinine Direct ELISA Kit. All 15 samples showed a presence of cotinine at a level greater than 500 ng/ml.

Three urine samples submitted by individuals exposed to passive inhalation for over 30 days all showed levels of 5 to 10 mg/mL of cotinine when extrapolated of a dose response curve.

Sensitivity
Assay sensitivity based on the minimum Cotinine concentration required to produce a three standard deviation from assay Ao is 1 ng/ml.

Specificity
The specificity of This Cotinine ELISA was determined by generating inhibition curves for each of the compounds listed below The antisera cross-reactivities below

Compound	Approx. ng/ml equivalent to 100 ng Cotinine/ml	Cross-reactivities
Cotinine	100	100
Nicotine	>10000	< 1
Nicotinamide	>10000	<1
Nicotinic Acid	>10000	<1

Cross-Reactivity with Unrelated Drugs
Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 50,000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level.

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine , Barbital, Butabarbital, Caffeine , Cocaine, Carbamazepine, Codeine , Chloroquine, Chloropromazine, Carbromal , Desipramine, Dextromethorphan, Dextropropoxyphene , 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide , Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone , Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytoin, Mephobarbital, Methyl PEMA, Methsuximide , 4-Methylprimidone, Morphine , Meperidine , Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital , Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline,Tetrahydrozoline

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