DRG® Serum Cotinine (EIA-3242)

Revised 30 Nov. 2009 (Vers. 2.0) **RUO in the USA**

Intended Use

For in vitro Use Only. In the United States, this kit is intended for Research Use Only.

The DRG Cotinine ELISA kit is intended for use in clinical and analytical laboratories.
It provides qualitative screening results for cotinine in human serum at a cut-off concentration of 25ng/mL.

This assay provides only a preliminary analytical test result. Clinical consideration and professional judgment must be applied to any substance abuse test result, particularly in evaluating a preliminary positive result. In order to obtain a more confirmed analytical result a more specific alternative chemical method is needed. Gas Chromatography/Mass Spectrometry (GC/MS) is the preferred confirmatory method.

Principle of the Test

The DRG Cotinine ELISA Kit is a competitive enzyme immunoassay for the detection of cotinine in human serum. The wells of the microtitre strips are coated with anti-cotinine antibody. During the first incubation, the horseradish peroxidase (HRP) labelled cotinine competes with the free cotinine in the patient sample for the anti-cotinine antibody binding sites on the microtitre strips. The wells are washed to remove any excess enzyme material prior to addition of the TMB substrate solution. Addition of the stop solution terminates the reaction and absorbances are read spectrophotometrically at 450nm.

Reagents

Anti-Cotinine Coated Plate – 1 plate [a]
12 x 8 well strips in break-apart format. Anti-cotinine polyclonal antibody immobilised on a polystyrene plate supplied in dry form. Contains BSA 0.001%.

Enzyme Conjugate – 15 mL [b]
Cotinine derivative labelled with horseradish peroxidase <0.1% (v/v) and diluted in a protein matrix with stabilisers. Contains preservatives.

Wash Buffer – 50 mL [c]
30 x concentrate, 0.1% (v/v) surfactant. Dilute each vial to 1500 mL with distilled water before use.

Substrate Solution – 20 mL [d]
One bottle containing <0.05% 3,3',5,5'-tetramethylbenzidine.

Stop Solution – 20 mL [e]
One bottle containing 1 mol/L sulphuric acid. Treat this solution as corrosive.

Negative Calibrator – 1 mL [f]
Protein matrix negative for cotinine.

Positive Calibrators – 1 mL each level
Protein matrix containing 10 ng/mL Cotinine [g]
Protein matrix containing 25 ng/mL Cotinine [h]

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Protein matrix containing 50 ng/mL Cotinine [i]
Contains preservatives.

Warning And Precautions

1. The handling of food or drink near the kit reagents is not recommended.
2. Any skin complaints, cuts or abrasions should be suitably protected.
3. 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.
4. Some of the assay reagents contain sodium azide which may react with copper or lead plumbing to form potentially explosive metal azides. When disposing of these reagents, always flush with a large volume of water to prevent azide build up.
5. Do NOT pipette reagents by mouth.
6. Do NOT add sodium azide to samples as a preservative!
7. Keep all containers closed when not in use to avoid microbial contamination.
8. Do NOT use reagents after the expiration date.
9. Do NOT mix reagents from different kits or manufacturers.
10. Do NOT freeze reagents.
11. It is suggested that all reagents be kept out of direct sunlight whenever possible.
12. Stop Solution is corrosive; handle with care.

Materials Required but not Provided

1. Positive and negative controls.
2. Automated microtitre plate reader with a 450nm filter, no temperature regulation required for the reader.
3. Precision pipettes with disposable tips. Use clean tips for each reagent to avoid contamination.
4. Automated microtitre plate washing machine, manual microtitre plate washer or 350µL eight-channel pipette for dispensing diluted wash buffer.
5. A timer for timing 30 minute intervals.
6. A clean 1.5L measuring cylinder for dilution of wash buffer concentrate.
7. Distilled or deionised water.

Specimen Collection and Storage

If the sample cannot be analysed immediately, store at 2-8°C for up to 28 days or at -20°C for longer storage.

Handle all specimens as if they were potentially infectious.

Storage and Stability of Reagents

Store all opened/unopened reagents at 2-8°C. The reagents are stable until the expiration date indicated on the reagent labels.
Surplus microtitre strips must be repackaged immediately in the resealable foil pouch with desiccant. Failure to follow the storage instructions may result in deterioration in the performance of the assay.

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Crystals may form in the wash buffer on storage. Ensure these are all transferred when diluting the wash buffer.
The substrate should be clear in colour. Any blue colouring indicates that the reagent has been contaminated and must be discarded. Do not expose the substrate to light.
Turbidity or precipitation in any kit component is an indication of deterioration and the component should be discarded.

Assay Procedure

Prepare Wash Buffer by diluting 1:30 in distilled water.

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 should be tested in duplicate.

1. Add 10 µL of sample, calibrator, or control to each well within 25 minutes.
2. Add 100 µL of Enzyme Conjugate to each test well.
3. Incubate for 30 minutes.
4. Wash the plate four times with 350 µL Wash Buffer using a plate washer.
5. Add 100 µL of Substrate Solution to each well and incubate for 30 minutes.
6. Add 100 µL of Stop Solution to each well.
7. Measure the absorbance at 450 nm within 15 minutes.

Quality Control

Calibrators should be included each time an assay is performed. Read the stopped assay within 15 minutes.

Controls are not supplied with the kit but users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.
The negative control must have an absorbance greater than the 25ng/mL calibrator.
The positive control must have an absorbance less than the 25ng/mL calibrator.
If the positive or negative controls do not have an absorbance less than or greater than the 25ng/mL calibrator respectively then the assay results are invalid. The assay should be repeated, if the control results are still out with the limits mentioned above contact Technical Services at DRG .

Interpretation of the Result

Positive Result

Any sample with an absorbance less than or equal to the 25ng/mL cut off calibrator is considered a positive. A positive result does not provide any information on the level of intoxication or concentration of the drug, it only indicates that the sample may contain drug above the cut-off level in qualitative terms.

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Negative Result
Any sample with an absorbance greater than the 25ng/mL cut off calibrator is considered a negative. A negative result does not necessarily indicate the absence of drug in the sample, it only indicates that the sample does not contain drug above the cut-off level in qualitative terms.

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

Limitations

A positive result from this assay should be confirmed by another generally accepted non-immunological method such as GC/MS. The test is designed for use with human serum only.
There is a possibility that other substances and/or factors not listed may interfere in the test and cause false results, eg technical or procedural errors.
In the event of deterioration in the analytical performance of the device or damage to the kit during transport please contact Technical Services at DRG.

Specificity

Non Cross reactants at 100,000ng/mL, relative to the cut-off concentration

6-Acetyl Morphine	**(-) Ephedrine**	**Nitrazepam**
7-Aminoflunitrazepam	(+) Ephedrine	Norbuprenorphine
Alprazolam	Fenfluramine	Norcodeine
Amitriptyline	Fentanyl	Nordiazepam
Amobarbital	Flunitrazepam	Norfluoxetine
Amphetamine	Fluoxetine	Nor-LSD
Anhydroecgonine	Flurazepam	Normorphine
Anhydroecgonine methyl ester	Heroin	Oxazepam
Ascorbic Acid	Hexobarbital	Oxycodone
Aspirin	Hydrocodone	Oxymorphone
Benzoylecgonine	Hydromorphone	Paracetamol
Buprenorphine	3-Hydroxyflunitrazepam	Pentobarbital
Butalbarbital	11-Hydroxy Δ9-THC	Phencyclidine
Caffeine	Ibuprofen	Pheniramine
Cannabidiol	Ketamine	Phenobarbital
Chlordiazepoxide	LAAM	β Phenylethylamine
Chloroquine	Lidocaine	Phentermine
Chlorpheniramine	Lorazepam	Phenylpropanolamine
Clonazepam	LSD	Pholcodeine
Cocaethylene	MBDB	Prazepam
Cocaine	MDA	Propoxyphene
Codeine	MDEA	Propranolol
Δ9-THC	MDMA	(-) Pseudoephedrine
Desalkylflurazepam	Meperidine	(+) Pseudoephedrine
Desmethylflunitrazepam	Methadone	Ranitidine
Dextromethorphan	Methamphetamine	Salicylate
Diazepam	Midazolam	Secobarbital
Dihydrocodeine	Morphine	Temazepam
Dothiepin	Morphine-3-Glucuronide	Tramadol
Ecgonine methyl ester	Nalorphine	Triazolam
EDDP	Naloxone	Tyramine
EMDP	11-nor-9-Carboxy- Δ9-THC	Warfarin

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Cross Reactants

Compounds / Cross Reactants	ng/mL	Apparent Cotinine ng/mL	% Reactivity
Nicotine	10,000	> 50	---
	1,000	< 10	----