Datasheet for ABIN1721161

Cotinine ELISA Kit

1 Validation

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Overview

Quantity:	96 tests
Target:	Cotinine
Reactivity:	Mouse, Rat
Method Type:	Competition ELISA
Minimum Detection Limit:	1 ng/mL
Application:	ELISA

Product Details

Purpose:	The Cotinine 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 is 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.
Sample Type:	Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The specificity of this Cotinine ELISA was determined by generating inhibition curves for each of the compounds listed below the antisera cross-reactivity below: h>Compoundd>Cotinine</tr>

	Approx. ng/mL equivalent to 100 ng Cotinine/mL	Cross-reactivity>	
	100	100	
	Nicotine	more than 10.000	less than 1
	Nicotinamide	more than 10.000	less than 1
	Nicotinic Acid	more than 10.000	less than 1

Cross-Reactivity (Details):	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, 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
Sensitivity:	Assay sensitivity based on the minimum Cotinine concentration required to produce a three standard deviation from assay Ao is 1 ng/mL.
Characteristics:	• This Cotinine Direct ELISA Kit is to be used with Mouse/Rat urine or serum. This assay has not tested for all possible applications. Cutoff criteria are important in deciding the sample dilution. • Specimens to which sodium azide has been added affect the assay.
Components:	1. Microwell coated with polyclonal Ab to Cotinine: 12 x 8 x 1 2. Standard Set (ready to use): 0.5 mL 3. Cotinine HRP Enzyme Conjugate (ready to use): 12 mL 4. TMB Substrate (ready to use): 12 mL 5. Stop Solution (ready to use): 12 mL
Material not included:	1. Distilled or deionized water 2. Precision pipettes

Product Details

3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

Target Details

Target:	Cotinine
Abstract:	Cotinine Products
Target Type:	Chemical
Background:	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 Calbiotech Cotinine Direct ELISA Kit is designed for the detection Cotinine in rat serum and urine. It can also be adapted for other fluids.
CAS-No:	486-56-6

Application Details

Application Notes:	The Calbiotech Cotinine Direct ELISA Kit is intended for the measurement of Cotinine in Mouse/Rat serum or urine.
Sample Volume:	100 µL
Assay Time:	2 - 3 h
Plate:	Pre-coated
Protocol:	All reagents must be brought to room temperature (18-26 °C) before use. • Pipette 10 µL of standards, controls and specimens into selected well in duplicate. • Add 100 µL of the Enzyme Conjugate to each well. Shake the plate, 10-30 s, to ensure proper mixing. • Incubate for 60 min at room temperature (18-26 °C) preferably in the dark. • Wash the wells 6 times with 300 µL distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. • 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. • Add 100 µL of Substrate reagent to each well.

Application Details

	• Incubate for 30 min at room temperature, preferably in the dark. • Add 100 µL of Stop Solution to each well. Shake the plate gently to mix the solution. • Read absorbance on ELISA Reader at 450 nm with in 15 min after adding the stopping solution.
Calculation of 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. 3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
Assay Precision:	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 ng/mL of Cotinine when extrapolated of a dose response curve.
Restrictions:	For Research Use only

Handling

Precaution of Use:	Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, recommended in the Centers for Disease Control/National Institutes of Health manual,
Handling Advice:	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.
Storage:	4 °C

Validation report #029687 for ELISA (ELISA)

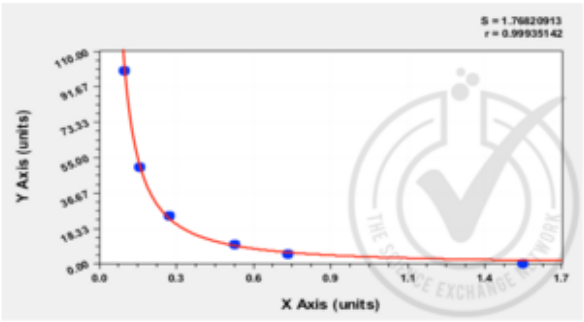
Successfully validated (ELISA (ELISA))

by Affina Biotechnologies, Inc

Report Number: 029687

Date: Apr 24 2014

Lot Number:	COT4179
Method validated:	ELISA (ELISA)
Positive Control:	Female rat serum spiked with cotinine
Negative Control:	Unspiked female rat serum
Notes:	Signal was detected in positive control sample and not in negative control sample.
Primary Antibody:	- Antigen: Cotinine - Catalog number: ABIN1721161 - Supplier: Calbiotech - Supplier catalog number: CO096D-100 - Lot number: COT4179
Controls:	• Positive control: Rat individual female serum (Biochemed, S128927) diluted 4-fold and then spiked with 50 ng/mL cotinine (Sigma, Cat#C5923). • Standard curve: 0, 5, 10, 25, 50 and 100 ng/mL cotinine provided in the ELISA kit • Spike control: identical to the positive control
Protocol:	• 10 µL of standard and samples were added to the 96-well strip plates provided in the kit and mixed with working solution of enzyme conjugate. All samples and standards were assayed in duplicate. • The microplate was covered and incubated at RT for 60 min. • Content of the wells was discarded and wells were washed 6 times with 250 µL of water. • 100 µL of substrate was added to each well. The plate was covered and incubated at RT for 30 min in the dark. • 100 µL of the Stop Solution was added per well. • The entire sample was transferred into a 96-well plate (Nunc, Maxisorp) • The optical density (OD value) of each well was read immediately using a microplate reader set to 450 nm. • The duplicate readings for each sample were averaged and the average zero standard optical density subtracted. The corrected average-value was tabulated as Average Absorbance. A standard curve was generated by plotting the mean OD value for each standard on the x-axis against the concentration on the Y-axis using Kaleidagraph. The concentration of samples was calculated using the CurveExpert v 1.4 logistic fit (Concentration = 3.52/(1-e-6.8*OD450).
Experimental Notes:	Nothing noted.



Validation image no. 1 for Cotinine ELISA Kit (ABIN1721161)

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample ng/ml	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Concentration (ng/mL)
Standard Curve	100	0.153	0.130	0.142	0.102	0.015749	100.3
	50	0.202	0.190	0.196	0.156	0.008398	48.9
	25	0.309	0.302	0.306	0.266	0.005002	24.0
	10	0.606	0.481	0.544	0.504	0.088228	11.4
	5	0.825	0.649	0.737	0.697	0.12411	8.00
	0	1.578	1.601	1.590	1.550	0.016369	3.5
Positive control	50	0.239	0.177	0.208	0.168	0.044	43.9
Negative Control	rat serum (diluted 4-fold)	1.927	1.923	1.925	1.885	0.003074	2.9

Validation image no. 2 for Cotinine ELISA Kit (ABIN1721161)

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. Value for Average Reading is derived from the average of two readings (OD 450nm). The Average Reading for blank sample (no conjugate added) was subtracted from all Average Readings to yield Average Absorbance values for Standards, spike controls and control samples. Standard deviation is included for all samples. The concentration of samples was calculated using the CurveExpert v 1.4 logistic fit (Concentration = 3.52/(1-e-6.8*OD450).