

<p>CALDON BIOTECH INC. PCP DIRECT ELISA KIT</p>

Catalog No. 208-192
(192 tests)

THE CALDON BIOTECH INC. (CBI) PCP DIRECT ELISA KIT IS INTENDED FOR FORENSIC USE ONLY.

The CBI PCP (Phencyclidine) Direct ELISA Kit provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GS-MS) is the preferred confirmatory method. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

EXPLANATION OF THE TEST

The CBI PCP Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of PCP in forensic samples like whole blood, serum, plasma and urine. PCP may be self administered either by smoking, inhalation, oral ingestion or injection. It is often mixed with other street drugs and used inadvertently. Free PCP and its metabolites are excreted in urine.

PRINCIPLES OF THE PROCEDURE

The CBI PCP 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 ul aliquot of a diluted unknown specimen is incubated with a 100 ul dilution of enzyme (Horseradish peroxidase) labeled PCP 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 500 pg/ml. The CBI PCP Direct ELISA Kit avoids extraction of urine or blood sample for measurement. It employs an PCP directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

MATERIALS AND METHODS

Materials and equipment required but not supplied with the CBI PCP Direct ELISA Kit are itemized below:

Materials

12x75 mm Disposable Glass or Plastic Tubes to predilute samples (if required).
Test Tube Racks.
Micropipets (single channel or multichannel) or automated pipetting stations.

Equipment

Refrigerator (for kit storage).
Interval Timer.
Wash bottle or Plate Washer.
Microplate reader capable of reading at 450 nm. And 650 nm.

REAGENTS PROVIDED

Component	192 Test Kit Cat#208-0192	480 Test Kit Cat#208-0480
96 well Micro-plate	2	5
PCP Conjugate	25 ml	55 ml
Positive Ref. Std	2 ml	5 ml
Neg Std	2 ml	5 ml
TMB Substrate	28 ml	55 ml
Stop Reagent	25 ml	55 ml

96 well micro-plate. The micro-plate is coated with polyclonal anti-PCP via a spacer chain to provide optimally oriented binding sites.

PCP-Enzyme Conjugate The conjugate solution contains a PCP derivative labeled with horseradish peroxidase in a stabilized protein buffer solution, pH 7.6 containing azide free preservatives

Positive Reference Standard. This contains 10 ng /ml of PCP dissolved in a synthetic urine containing azide free preservatives. This is to be diluted to the laboratory cutoff.

Normal Control. This bottle contains drug free synthetic urine 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.

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 dilute acid.
 13. Bring all reagents to room temperature.
 14. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
- 1) Dilute forensic specimens, to the necessary range with Phosphate Buffer Saline pH 7.0 . (Urine samples are normally diluted 1:5 for cutoff of 25 ng/ml of PCP.) The dilution factor can be adjusted based on the laboratories cutoff.
 - 2) Add 10 µl. of calibrators and standards to each well in duplicate.
 - 3) Add 10 µl. of the diluted specimens in duplicate (recommended) to each well.
 - 4) Add 100 µl. of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
 - 5) Incubate for 60 minutes at room temperature (20-25 ° C) preferably in the dark, after addition of enzyme conjugate to the last well.
 - 6) Wash well 6 times with 350 µl distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells.
 - 7) 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.
 - 8) Add 100 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
 - 9) Incubate for 20 minutes at room temperature, preferably in the dark.
 - 10) Add 100 µl of Stop Solution to each well, to change the blue color to yellow.
 - 11) Measure the absorbance at a dual wavelength of 450 nm. and 650 nm. Compare average absorbance readings obtained from each unknown specimen with the average absorbance obtained from the Positive Reference Standard.
 - 12) Wells should be read within 2 hours of yellow color development.

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 degrees centigrade.

SPECIMEN COLLECTION

Precautions.

The CBI PCP Direct ELISA Kit is to be used with human forensic samples, like whole blood, serum and plasma. CBI has not tested all possible applications of this assay. **The Cutoff criteria is important in deciding the sample dilution.**

Additives.

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

Interfering Substances.

There are no commonly abused drugs which alter the values obtained with the CBI PCP Direct ELISA Kit.

Storage and Handling Instructions.

Urine samples should be stored at 2 - 4 degrees centigrade until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

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 .

The following data represent a typical dose/response curve.

PCP (ng/ml)	Absorbance
0	1.534
2	0.674
5	0.416
10	0.211

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 PCP. If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called NEGATIVE for PCP.

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.

INTERPRETATION

PCP and its metabolites appear in urine within hours after drug use and may persist for days. Thus a positive result documents PCP use. GC/MS is recommended for confirmation.

SPECIFIC**CHARACTERISTICS**Accuracy

75 whole blood samples and 35 urine samples collected from presumed non-users were tested in the CBI PCP Direct ELISA Kit . One hundred percent of these normal samples measured negative at 5 ng/ml for whole blood and 25 ng/ml for urine. Twenty whole blood samples which were previously confirmed positive for PCP by GC-MS employing a cut-off of 5 ng/ml, were tested in the CBI PCP Direct ELISA Kit . 19 of the samples were found to be positive i.e. above the cut-off of 5 ng/ml.

Precision

Intra-assay Precision

PCP C.V.% (ng/ml)	Mean Abs.	S.D.	
0	1.655	0.104	6.3
2	0.649	0.058	8.9
5	0.401	0.039	9.7
10	0.225	0.031	
13.8			

Sensitivity

Assay sensitivity based on the minimum PCP concentration required to produce a four standard deviation from assay A_0 is 500 pg/ml.

Specificity

The specificity of the CBI ELISA for PCP was determined by generating inhibition curves for

each of the compounds listed below The antisera cross-reactivities are listed below.

Compound ng/ml	%	Approx equivalent to Cross 5 ng PCP/ml reactivity
Phencyclidine (PCP)	100	5
1-[1-(2-thienyl)cyclohexyl]-piperidine	10	50
1-[1-(2-thienyl)cyclohexyl]-morpholine	10	50
1-(1-phenylcyclohexyl)pyrrolidine	10	50
1-(1-phenylcyclohexyl)morpholine	8	60

Cross-Reactivity with Unrelated Drugs

A human urine matrix was spiked with the following compounds at a concentration of 2000 ng/ml. None of these compounds gave values in the assay that are equal to or greater than the assay sensitivity level (0.5 ng/ml).

Amphetamine , Amobarbital, Barbitol, Butobarbital, Caffeine , Cocaine, delta-9-THC , 9-carboxy-THC, Benzoyllecgonine, Carbamazepine, Codeine , Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Ethosuximide, Ethotoin, Glutethimide , Hexobarbital, Lidocaine, Methadone-Primary, Methaqualone , Methadone Metabolite, Metharbital, Mephentoin, Methyl-x-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine , Meperidine, Methamphetamine, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA , Primidone

REFERENCES

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