SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765   Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

PCP Direct ELISA

Catalog Number **SE120101**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The PCP Direct ELISA is a specific and sensitive *In vitro* test to detect the presence of PCP (Phencyclidine) in samples such as whole blood, oral fluids, 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.

The PCP Direct ELISA 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 a100 μL dilution of enzyme (Horseradish peroxidase) labeled PCP derivative in microplate 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 PCP Direct ELISA avoids extraction of urine or blood samples for measurement. It employs a PCP directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene microplate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size, reducing matrix effects and interference with binding protein(s) or other macromolecules.

The PCP Direct ELISA 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.[3] Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Components

Materials Provided	96 Tests
Microwells coated with polyclonal anti-PCP	12 x 8 x 1
PCP Conjugate	12 mL
Immunalysis Positive Ref. Std	2 mL
Neg. Std	1 mL
TMB Substrate	12 mL
Stop Reagent	11 mL

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
1. The PCP Direct ELISA is to be used with human samples, such as whole blood, oral fluids, serum, urine, and plasma.
2. All possible applications of this assay have not been tested. Cutoff criteria are important in deciding the sample dilution.
3. Specimens to which sodium azide has been added affect the assay.

Storage/Stability

Store the kit at 2–8 °C. 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.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

The procedure as described may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipettor.

All reagents must be brought to room temperature (18–26 °C) before use.

1. Dilute specimens, to the necessary range with Phosphate Buffered Saline, pH 7.0. (Urine samples are normally diluted 21-fold for a PCP cutoff of 25 ng/mL.) The dilution factor and volume added can be adjusted based on the laboratory's cutoff.
2. Add 10 μL of appropriately diluted calibrators and standards to each well in duplicate (recommended).
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 (18–26 °C) preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash the wells 6 times with 350 μL of distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amounts of hemoglobin (some postmortem samples), use 10 mM Phosphate Buffered Saline, pH 7.0–7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.
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 30 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.
12. Wells should be read within 1 hour of yellow color development.

Results

If the average sample absorbance is equal to or less than the average absorbance of the laboratory positive reference standard the sample is <u>POSITIVE</u> for PCP.

If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called <u>NEGATIVE</u> 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.

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

<u>Note</u>: The dose/response curve shown 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.

References

1. Drugs on the Job. Time Magazine, March 17, 1986
2. Way, E.L., and Adler, T.K., Bull. Wld. Hlth. Org. 27:359 (1962)
3. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph, 73,1986.
4. Baselt, R.C., in : Advances in Analytical Technology, Vol.1., Baselt, R.C., (ed)., Biomedical Publications, (Foster City, CA) 112- 116.

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