

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

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

REFERENCES

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph. 73: 95-97 (1986).
2. N.Weiner. Norepinephrine, epinephrine and the sympathomimetic amines. In: The Pharmacological Basis of Therapeutics. 7th ed. p.145-180 (New York: MacMillan 1985).
3. J. Caldwell and P.S. Sever. The Biochemical Pharmacology of Abused Drugs. Clinical Pharmacology and Therapeutics. 16: 625- 638 (1974).
4. R. C. Baselt. In: Advances in Analytical Toxicology, Vol.1. p.87 - 93. Ed. R. C. Baselt, Biomedical Publications, Foster City, CA (1984).

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For Research Use Only. Not for use in Diagnostic Procedures.

**Amphetamine Direct ELISA**

Catalog No. AM080D (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) Amphetamine 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 (1). Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

SUMMARY AND EXPLANATION

The CBI Amphetamine Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of d-amphetamine in samples such as whole blood, oral fluids, serum, plasma and urine. While the assay will detect amphetamine use, interference by l-amphetamine and pseudo-ephedrine is virtually nonexistent. Amphetamine is a potent central nervous system stimulant. The (+)-isomer also referred to as d-amphetamine is three to four times more potent than the (-)-isomer, l-amphetamine (2). Amphetamine may be metabolized and excreted as the p-hydroxy isomer. Amphetamines act by inducing euphoria, irritability, anxiety and paranoia. Urinary excretion rates are influenced by the urinary pH with acidic urine favoring the excretion of unchanged drug(2). Up to 80% of a given dose may be excreted unchanged, especially in acid urine. Alkaline urine reduces the excretion of unchanged amphetamine to less than 5% of the dose.

PRINCIPLES OF THE TEST

The Amphetamine Direct ELISA Kit (for d-amphetamine measurement) 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 d-amphetamine 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. The Amphetamine Direct ELISA Kit avoids extraction of urine sample for measurement. It employs a d-amphetamine 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 allows an extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

Cat#: AM080D (192 tests)

For Order and Inquiries, please contact

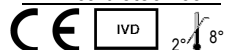


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MATERIALS PROVIDED	96 Tests
1. Microwells coated with polyclonal anti-d-amphetamine	12x8x1
2. d-Amph-Conjugate	12.5 ml
3. Positive Ref. Std	1ml
4. Neg Std	1 ml
5. TMB Substrate	14 ml
6. Stop Reagent	12.5 ml

MATERIALS 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. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactory until the expiration date is stored in the refrigerator at 2-40° C.
2. Store the kit at 2-8° C.
3. Keep microwells sealed in a dry bag with desiccants.
4. The reagents are stable until expiration of the kit.
5. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

1. 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, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984

2. This test kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. The Amphetamine Direct ELISA Kit is to be used with human samples, such as whole blood, oral fluids, serum, urine and plasma. has not tested all possible applications of

this assay. Cutoff criteria are important in deciding the sample dilution.

2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2 - 40 C until use. Samples should be well mixed before assay.
4. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

ASSAY PROCEDURE

All reagents must be brought to room temperature (18-26° 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.

- 1) Dilute specimens, to the necessary range with Phosphate Buffered Saline pH 7.0. (Urine samples 1:20 for a cutoff level of 500ng/ml.) The dilution factor can be adjusted based on the laboratories cutoff.
- 2) Add 10ul of appropriately diluted calibrators and standards to each well in duplicate.
- 3) Add 10ul of the diluted specimens in duplicate (recommended) to each well.
- 4) Add 100ul 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 preferably in the dark (18-26° C), after addition of enzyme conjugate to the last well.
- 6) Wash well 6 times with 350ul distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amount of hemoglobin (some postmortem samples) use 10mM 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 100ul 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 100ul 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 hours of yellow color development.

The following data represent a typical dose/response curve.

d-amphetamine (ng/m)	Absorbance
0	2.459
10	0.891
25	0.431
50	0.255

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.