

Product Information

Amphetamine Direct ELISA

Catalog Number **SE120144**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The 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 pseudoephedrine 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 derivative. 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 acidic urine. Alkaline urine reduces the excretion of unchanged amphetamine to less than 5% of the dose.

The Amphetamine 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 µL aliquot of a diluted unknown specimen is incubated with 100 µL of diluted enzyme (horseradish peroxidase) HRP labelled d-amphetamine 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 1 ng/mL.

This kit avoids extraction of urine samples for measurement. It employs a d-amphetamine 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 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.

Components

Materials Provided	96 Tests
Microwells coated with polyclonal anti-d-amphetamine	12 x 8 x 1
d-amphetamine-Conjugate	12 mL
Immunoanalysis Positive Reference Standard	2 mL
Negative Standard	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 or disposable pipette tips
3. ELISA reader capable of reading absorbance at 450 nm
4. Absorbent paper or paper towel
5. 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 Amphetamine Direct ELISA is to be used with human samples, such as whole blood, oral fluids, serum, urine, and plasma. It has not been tested in 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 2–8 °C until use. Samples should be well mixed before assay.
4. Avoid multiple freeze-thaw cycles.
5. Urine samples should be shipped refrigerated in ice.

Storage/Stability

Store the kit at 2–8 °C. The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactory until the expiration date when stored in the refrigerator 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.

It is recommended that serum samples be run in duplicate.

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.

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 500 ng/mL.) The dilution factor can be adjusted based on the laboratories cutoff.
2. Add 10 µL of appropriately diluted 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 preferably in the dark (18–26 °C), after addition of enzyme conjugate to the last well.
6. Wash 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 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 (18–26 °C), preferably in the dark.
10. Add 100 µL of Stop Solution to each well, to change the color from blue 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 development of yellow color.

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.

The following data represent a typical dose/response curve.

d-amphetamine (ng/mL)	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 cut-off calibrator should be run with every plate.

References

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

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