Product Information

Methamphetamine ELISA

Catalog Number SE120077
Storage Temperature 2–4 °C

TECHNICAL BULLETIN

Product Description
The Methamphetamine ELISA kit is a specific and sensitive in vitro test to detect the presence of d-methamphetamine in samples such as whole blood, oral fluids, serum, plasma, and urine. While the assay will detect amphetamine use, interference by l-methamphetamine and pseudoephedrine is virtually nonexistent. Methamphetamine is a potent central nervous system stimulant with less peripheral actions than amphetamine. The (+)-isomer also referred to as d-methamphetamine, is ten times more potent than the (−)-isomer (l-methamphetamine). Amphetamines act by inducing euphoria, irritability, anxiety, and paranoia. Methamphetamine is metabolized to its active metabolite amphetamine via N-demethylation and is further metabolized by hydroxylation and deamination of amphetamine. Urinary excretion rates are influenced by the urinary pH with acidic urine favoring the excretion of unchanged drug. Alkaline urine reduces the excretion of unchanged methamphetamine to less than 5% of the dose.

The Methamphetamine ELISA Kit (for d-methamphetamine 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-methamphetamine 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.

The Methamphetamine ELISA Kit avoids extraction of urine sample for measurement. It employs a d-methamphetamine 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 proteins(s) or other macromolecules.

The Methamphetamine 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 (GC-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.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
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<tbody>
<tr>
<td>Microwells coated with polyclonal anti-d-methamphetamine</td>
<td>12 x 8 x 1</td>
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<tr>
<td>d-Meth-Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Immunalysis Positive Ref. Std</td>
<td>2 mL</td>
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<tr>
<td>Neg Std</td>
<td>1 mL</td>
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<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
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<tr>
<td>Stop Solution</td>
<td>11 mL</td>
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Reagents and Equipment Required but Not Provided.
1. 12 x 75 mm disposable glass or plastic culture tubes to pre-dilute samples (if required).
2. Manual or electronic micropipettes (single channel or multichannel) or automated pipetting stations.
3. Refrigerator (for kit storage).
4. Interval Timer.
5. Wash bottle or Plate Washer.
6. Microplate reader capable of reading at 450 nm and 650 nm.
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 Methamphetamine ELISA kit is to be used with human samples, such as whole blood, oral fluids, serum, urine, and plasma. All possible applications of this assay have not been tested. 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 2–4 °C until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with ice packs or equivalent.

Storage/Stability
The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2–4 °C.

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.

All reagents must be brought to room temperature (18–26 °C) before use. The procedure as described 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 are normally diluted 1:20 for a methamphetamine cutoff of 500 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 appropriate wells in duplicate.
3. Add 10 µL of the diluted specimens in duplicate (recommended) to appropriate wells.
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 POSITIVE for methamphetamine.

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

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.

<table>
<thead>
<tr>
<th>d-methamphetamine (ng/mL)</th>
<th>Absorbance</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.519</td>
</tr>
<tr>
<td>10</td>
<td>0.649</td>
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<tr>
<td>25</td>
<td>0.471</td>
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<tr>
<td>50</td>
<td>0.359</td>
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References