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



Cocaine Metabolite Direct Elisa (Benzoylcegonine Specific)

Catalog No. CO084D (96 Tests)

INTENDED USE


The Calbiotech, Inc. (CBI) Cocaine Metabolite Direct ELISA Kit is a specific and sensitive in-vitro test to detect the presence of Benzoylcegonine (BE) in forensic samples such as whole blood, serum, plasma and urine.

SUMMARY AND EXPLANATION

Cocaine abuse is widespread and its prevalence may be increasing in all social and age strata (1). The drug is generally inhaled or smoked (1,2). Several methods for measurement of Cocaine Metabolite in urine exist (3-6). Benzoylcegonine, a major metabolite appears within minutes in urine (3). Since the number and proportion of metabolites vary in subjects, results are expressed in benzoylcegonine equivalents per ml. The Cocaine Metabolite Direct ELISA Kit is a single incubation assay providing results similar to those obtained by existing methods (4-6). Native (unaltered) cocaine urine concentration is far lower than that of its major metabolite benzoylcegonine. After intra- venous administration of 100mg cocaine urine concentrations ranged from 1.2 - 2.4 ug/ml compared with concentrations ranging from 5 - 55 ug/ml for benzoylcegonine (3). Cocaine was undetectable (at a 50 ng/ml cut-off) 12 hours after administration in comparison with benzoylcegonine which persists hours to days after administration (7). It has been suggested that a benzoylcegonine/cocaine ratio of less than 100 is indicative of use within the past 10 hours (7).

PRINCIPLE OF THE TEST

The Cocaine Metabolite 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 a 100 µl. dilution of enzyme (Horseradish peroxidase) labeled Benzoylcegonine 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 Cocaine Metabolite Direct ELISA Kit avoids extraction of urine or blood sample for measurement. It employs an Benzoylcegonine 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#: CO084D (96 Tests)
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MATERIALS PROVIDED		96 Tests
1.	Microwells coated with polyclonal anti-Benzoylcegonine	12x8x1
2.	Benzoylcegonine Conjugate	12.5 ml
3.	Positive Reference Standard	1 ml
4.	Negative Standard	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 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. 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, 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. Not for use in diagnostic procedures.
3. 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Dilute forensic specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (Urine samples are normally diluted 1:10 for a Benzoylcegonine cutoff of 300 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.
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. 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.

Example of a Standard Curve

The following data represent a typical dose/response curve.

Benzoylcegonine (ng/ml)	Absorbance
0	2.045
10	1.455
25	0.866
50	0.584

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.