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Product Information

Cocaine/Benzoylecygonine Direct ELISA

Catalog Number **SE120036**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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 metabolites in urine exist.[3-6] Benzoylecgonine, a major metabolite, appears within minutes in urine.[3] Since the number and proportion of metabolites vary in subjects, results are expressed in benzoylecgonine equivalents per ml. The Cocaine/ Benzoylecgonine 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 benzoylecgonine. After intravenous administration of 100 mg of cocaine, urine concentrations ranged from 1.2–2.4 μg/ml compared with concentrations ranging from 5–55 μg/ml for benzoylecgonine.[3] Cocaine was undetectable (at a 50 ng/ml cut-off) 12 hours after administration in comparison with benzoylecgonine which persists hours to days after administration.[7] It has been suggested that a benzoylecgonine/cocaine ratio of less than 100 is indicative of use within the past 10 hours.[7]

The Cocaine/Benzoylecgonine 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 benzoylecgonine 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 Cocaine/Benzoylecgonine Direct ELISA Kit avoids extraction of urine or blood samples for measurement. It employs a benzoylecgonine 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 Cocaine/Benzoylecgonine Direct ELISA Kit is a specific and sensitive *in vitro* test to detect the presence of benzoylecgonine (BE) in forensic samples such as whole blood, serum, plasma, and urine.

Components

Materials Provided	96 Tests
Microwells	12 × 8 × 1
BE-Conjugate	12 ml
Immunalysis Positive Reference Standard	2 ml
Negative Standard	1 ml
TMB Substrate	12 ml
Stop Reagent	11 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- 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 Cocaine/Benzoylecgonine Direct ELISA Kit is to be used with human forensic samples, like whole blood, serum, 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 2–8 °C until use. Samples should be well mixed or vortexed before assay.
4. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with ice packs or equivalent.

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 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 diluted standards and diluted specimens 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 and gently mix.

1. Dilute forensic specimens, to the necessary range with Phosphate Buffered Saline, pH 7.0. (Urine samples are normally diluted 1:10 for a Benzoylecgonine 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 standards into selected wells. It is recommended standards be run in duplicate.
3. Add 10 µl of the diluted specimens into selected wells. It is recommended samples be run in duplicate.
4. Add 100 µl of the BE-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 BE-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 non-specific 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 residual BE-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 TMB 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

The following data represent a typical dose/response curve.

Benzoylecgonine (ng/ml)	Absorbance
0	2.2
10	0.52
25	0.33
50	0.27

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.

References

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph. **73**, 95-97 (1986).
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