

**Cross-Reactivity with Unrelated Drugs**

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 2000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (<1 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbitol, Butabarbital, Caffeine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbolmal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5- Diphenylhydantoin, 10-11- Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone - primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytoin, a-Methyl-a-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH

**REFERENCES**

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**Warning**

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# BIO-QUANT

## Cocaine Metabolite Direct Elisa (Benzoylcegonine Specific)

Catalog No. BQ 212-096 (96 tests)

**INTENDED USE**

The 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

MATERIALS PROVIDED		96 tests
1.	96 wells Micro-plate	12x8x1
2.	BE-Conjugate	12.5 ml
3.	Immunalysis 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 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. **Precautions**  
The Benzoyllecgonine Direct ELISA Kit is to be used with human forensic samples, like whole blood, serum and plasma. has not tested all possible applications of this assay. Cutoff criteria are important in deciding the sample dilution.
2. **Additives**  
Specimens to which sodium azide has been added affect the assay.
3. **Storage and Handling Instructions**  
Urine samples should be stored at 2 – 40 C until use. Samples should be well mixed or vortexed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

**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 Benzoyllecgonine 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.

The following data represent a typical dose/response curve.

Benzoyllecgonine (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.

**SPECIFIC PERFORMANCE CHARACTERISTICS****1. Accuracy**

60 whole blood samples and 35 urine samples collected from presumed non-users were tested in the Cocaine Metabolite Direct ELISA Kit . One hundred percent of these normal samples measured negative at 50 ng/ml of Benzoyllecgonine for whole blood and 300 ng/ml of Benzoyllecgonine for urine. Thirty five whole blood samples which were previously confirmed positive for Benzoyllecgonine by GC-MS employing a cut-off of 50 ng/ml, were tested in the Cocaine Metabolite Direct ELISA Kit. All the samples were found to be positive i.e. above the cut-off of 50 ng/ml.

**2. Precision**

The precision of the Cocaine Metabolite Direct ELISA Kit has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.

**3. Intra-assay Precision**

Intra-assay precision was determined with reference controls. A 0,10,25 and 50 ng/ml standard was assayed five times in the same assay.

Benzoyllecgonine (ng/ml)	Mean Abs.	S.D.	C.V.%
0	2.167	0.173	8.0
10	1.117	0.129	11.55
25	0.605	0.045	7.4
50	0.418	0.033	7.8

**4. Sensitivity**

Assay sensitivity based on the minimum Benzoyllecgonine concentration required to produce a four standard deviation from assay A<sub>0</sub> is 1 ng/ml.

**5. Specificity**

The specificity of the Cocaine Metabolite ELISA was determined by generating inhibition curves for each of the compounds listed below

Compound	Approx. ng/ml equivalent to 50ng	Cross-reactivity
Benzoyllecgonine	50	100
Cocaine	750	6.66
Cocaeethylene	>5000	<1
Norcocaine	>5000	<1
Ecgonine	>5000	<1
Ecgonine Methyl	>10000	<0.5
Ester	>10000	<0.5