BECKMAN COULTER®

Emit® II Plus
Cocaine Metabolite Assay

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Catalog Number	Product Description	Quantity/ Volume
OSR9H229	**Emit® II Plus Cocaine Metabolite Assay**	
	OSR9H618 R1 (Antibody/Substrate Reagent 1)	2 x 29 mL
	OSR9H648 R2 (Enzyme Reagent 2)	2 x 14 mL

1 INTENDED USE

The Emit® II Plus Cocaine Metabolite Assay is a homogeneous enzyme immunoassay with a 150 ng/mL or 300 ng/mL cutoff (SAMHSA initial test cutoff level).[1] The assay is intended for use in the qualitative and semiquantitative analyses of benzoylecgonine (cocaine metabolite) in human urine. Emit® II Plus assays are designed for use on a variety of AU® Clinical Chemistry Systems (see Section 6, Instruments).

The Emit® II Plus Cocaine Metabolite Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[2] Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 SUMMARY AND EXPLANATION OF THE TEST

Cocaine is a central nervous system stimulant that is extracted from the coca plant. As a drug of abuse, cocaine is self-administered in a variety of ways, including inhalation and intravenous injection. Cocaine base can be smoked in a form that is commonly known as "crack." Cocaine is rapidly absorbed, especially when smoked. While all forms are potentially addicting, "crack" is especially likely to lead to dependence because of its more rapid and heightened effect on the abuser.[2]

Excretion rate patterns vary with the mode of administration and from individual to individual. Cocaine is almost completely metabolized, primarily in the liver, with only about one percent excreted in the urine unchanged. Most cocaine is eliminated as benzoylecgonine, the major metabolite of cocaine. Cocaine is also excreted in relatively lesser amounts as ecgonine methyl ester and ecgonine. Cocaine metabolites may be detected in urine for up to a couple of days after cocaine is used. Benzoylecgonine can be detected in urine within four hours after cocaine inhalation and remain detectable in concentrations greater than 1000 ng/mL for as long as 48 hours.[3–6]

The Emit® II Plus Cocaine Metabolite Assay tests for benzoylecgonine, the major metabolite of cocaine, in human urine. Positive results for specimens containing other compounds structurally unrelated to benzoylecgonine have not been observed.

Methods historically used for detecting benzoylecgonine in biological fluids include high-performance liquid chromatography, gas-liquid chromatography, and enzyme immunoassay.[7–9]

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.

3 PRINCIPLE

The Emit® II Plus Cocaine Metabolite Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[10] The assay is based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the specimen can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial (*Leuconostoc mesenteroides*) enzyme employed in the assay.

4 REAGENTS

Reagents contain the following substances:

Antibody/Substrate Reagent 1

Sheep polyclonal antibodies* to benzoylecgonine (2.2 µg/mL), bovine serum albumin, G6P (15 mM), NAD (12 mM), preservatives, and stabilizers

Enzyme Reagent 2

Benzoylecgonine labeled with bacterial G6PDH (0.46 U/mL),* HEPES buffer, bovine serum albumin, preservatives, and stabilizers

****The antibody titer and enzyme conjugate activity may vary from lot to lot.***

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

Precautions:

- For *in vitro* diagnostic use.
- Reagent 1 contains nonsterile sheep antibodies.
- Reagent 2 contains nonsterile mouse monoclonal antibodies
- Reagents 1 and 2 contain nonsterile bovine serum albumin.

Note: Caps must always be replaced on the original containers.

Preparation of Reagents:

The Emit® II Plus Cocaine Metabolite Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. Close the reagent bottles when not in use.

Storage of Assay Components:

When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Refer to the application sheet for on instrument stability information. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C. **Improper storage of reagents can affect assay performance.**

5 SPECIMEN COLLECTION AND PREPARATION

- Urine specimens may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics can adsorb certain drugs.
- If not analyzed immediately, specimens may be stored unrefrigerated for up to 7 days following collection. After 7 days, specimens should be stored frozen.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine specimens within the pH range of 3.0 to 11.0 do not require prior adjustment of pH. However, benzoylecgonine in specimens can degrade upon prolonged exposure to pH levels greater than 9.0.
- Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.
- Human urine specimens should be handled and treated as if they are potentially infectious.

6 PROCEDURE

Materials Provided

Emit® II Plus Cocaine Metabolite Assay
Antibody/Substrate Reagent 1
Enzyme Reagent 2

Materials Required But Not Provided

Emit® Calibrators/Controls

9A509UL	Level 0 (0 ng/mL)
9A549UL	Level 2 (150 ng/mL)
9A569UL	Level 3 (300 ng/mL)
9A589UL	Level 4 (500 ng/mL)
9A609UL	Level 5 (1000 ng/mL)

Materials Required But Not Supplied

Commercial controls (see Quality Control, Semiquantitative Analysis)

Instruments

Beckman Coulter customers and Siemens Healthcare Diagnostics customers contact their respective technical assistance centers for application sheets for specific AU Clinical Chemistry Systems. Refer to the Instrument User's Guide for appropriate checks and maintenance instructions.

Calibration

Note: These reagents are qualified for use with these calibrators only. However, other control material may be used for quality control purposes.

Table 1 — Emit® Calibrators/Controls for Use in Qualitative or Semiquantitative Analysis

Desired Cutoff Level (ng/mL)	Additional Recommended Calibrators/Controls for Qualitative Analysis (ng/mL)	Required Calibrators/Controls for Semiquantitative Analysis (ng/mL)
150 (Level 2)	Level 0 (0) Level 5 (1000)	Level 0 (0) Level 2 (150) Level 3 (300) Level 4 (500) Level 5 (1000)
300 (Level 3)	Level 0 (0) Level 5 (1000)	

Note: For any individual cutoff level, a calibrator/control is used as either a calibrator or as a control when the assay is used for qualitative analysis. When a calibrator/control is used as a calibrator for an individual cutoff level, the other level calibrators/controls (above or below it, as listed above) are used as controls.

Qualitative Analysis

Calibrate by running the appropriate Emit® Calibrator/Control Level for the desired cutoff listed in Table 1 in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the application sheet for additional information and instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis

Prepare a calibration curve by running the appropriate Emit® Calibrators/Controls listed in Table 1. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the application sheet for additional information and instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

Validate the calibration by assaying controls. Ensure that the result from Emit® Calibrator/ Control Level 0 (0 ng/mL) or Emit® Calibrator/Control Level 5 (1000 ng/mL) relates appropriately to the result from the cutoff calibrator chosen from column 1 in Table 1. That is,

- If Emit® Calibrator/Control Level 0 (0 ng/mL) was run, ensure that the result is negative relative to the selected cutoff calibrator level.
- If Emit® Calibrator/Control Level 5 (1000 ng/mL) was run, ensure that the result is positive relative to the selected cutoff calibrator level.

Once the calibration is validated, run urine specimens.

Semiquantitative Analysis

Validate the calibration curve by assaying commercial controls. Ensure that control results fall within acceptable limits as defined by your laboratory.

Once the calibration curve is validated, run urine specimens.

Note: Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

7 RESULTS

Qualitative Analysis

Refer to Table 1 for the appropriate cutoff Emit® Calibrator/Control. Table 1 contains the concentration of benzoylecgonine present in the selected Emit® Calibrator/Control selected as a cutoff for distinguishing "positive" from "negative" specimens.

Positive Results. A specimen that gives a change in rate value greater than or equal to the Emit® Calibrator/Control cutoff rate value is interpreted as positive.

Negative Results. A specimen that gives a change in rate value less than the Emit® Calibrator/Control cutoff rate value is interpreted as negative: Either the specimen does not contain cocaine metabolites or cocaine metabolites are present in concentrations below the cutoff level for this assay.

Semiquantitative Analysis

The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for confirmation by GC/MS. Semiquantitation also permits the laboratory to establish quality control procedures and assess control performance. Refer to the Analytical Recovery section for the semiquantitative range.

Using the Emit® II Plus Cocaine Metabolite Assay, it is possible to make semiquantitative determinations of benzoylecgonine. An estimate of relative total drug concentrations may be obtained by running the appropriate Emit® Calibrators/Controls: Levels 0 (0 ng/mL), 2 (150 ng/mL), 3 (300 ng/mL), 4 (500 ng/mL), 5 (1000 ng/mL). Refer to the application sheet for instructions.

8 LIMITATIONS OF THE PROCEDURE

- The assay is designed for use with human urine only.
- A positive result from the assay indicates the presence of cocaine metabolites but does not indicate or measure intoxication.
- Boric acid is not recommended as a preservative for urine.
- There is a possibility that substances and/or factors not listed (e.g., technical or procedural errors) may interfere with the test and cause false results.
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- Immunoassays that produce a single result in the presence of a drug and its metabolites cannot fully quantitate the concentration of individual components.

9 EXPECTED VALUES

When the Emit® II Plus Cocaine Metabolite Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given specimen cannot be estimated. The assay results distinguish between positive and negative specimens-positive indicating specimens that contain cocaine metabolites.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the metabolites detected by the assay (see Section 7, Results).

10 PERFORMANCE

The data appearing in this section were collected on the AU400®/AU600® Clinical Chemistry System using the Emit® II Plus Cocaine Metabolite Assay. Results are current at the date of publication; however, results may vary due to analyzer-to-analyzer differences. Beckman Coulter customers and Siemens customers contact their respective technical assistance centers for additional information. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Method Comparison

Qualitative Results

150 ng/mL CUTOFF

One hundred twenty-five (125) samples were analyzed by the Emit® II Plus Cocaine Metabolite Assay and on the SYVA®-30R Biochemical System. Sixty two (62) samples showed positive results by both methods, while fifty-seven (57) samples showed negative results by both methods.

The results from the AU600 are listed below along with the percent agreement with the SYVA®-30R. Discordant specimens contained benzoylecgonine by GC/MS analysis ranging from 89 ng/mL to 164 ng/mL at the 150 ng/mL cutoff. Discordant specimens contained benzoylecgonine by GC/MS analysis ranging from 283 ng/mL to 321 ng/mL at the 300 ng/mL cutoff. Data are summarized in Table 2.

Table 2 — Summary of Method Comparison

Assay	Positive	Negative	Agreement
Cocaine Metabolite 150	62	57	95.2 %
Cocaine Metabolite 300	37	85	97.6 %

Analytical Recovery

Negative human urine specimens were spiked with concentrations of benzoylecgonine.

Qualitative analysis of the specimens spiked with drug concentrations lower than the cutoff concentration were correctly identified as negative 100% of the time. Specimens spiked with drug concentrations greater than the cutoff were correctly identified as positive 100% of the time.

Negative human urine was spiked with concentrations of benzoylecgonine at levels of 45 to 750 ng/mL. Semiquantitative results are shown in Table 3.

Table 3 — Analytical Recovery of Semiquantitative Results

Concentration (ng/mL)	Mean (ng/mL)
100	94
225	244
270	288
330	311
450	470
750	833

Precision

Within run precision was calculated according to NCCLS Guideline EP5-A by running 2 replicates of the cutoff Calibrator/Control and positive and negative controls twice a day for 20 days (N=80). Total precision was also calculated from these data. The following data are presented in mAU/min.

Table 4 — Within-Run and Total Precision at 150 ng/mL

	Within Run Precision			Total Precision		
	Cutoff Cal.	Control 75%	Control 125%	Cutoff Cal.	Control 75%	Control 125%
Mean	314	293	329	314	293	329
SD	2.1	2.2	2.8	3.6	3.4	4.3
CV%	0.6	0.6	0.7	1.0	1.0	1.1

Table 5 — Within-Run and Total Precision at 300 ng/mL

	Within Run Precision			Total Precision		
	Cutoff Cal.	Control 75%	Control 125%	Cutoff Cal.	Control 75%	Control 125%
Mean	393	283	402	393	283	402
SD	3.5	1.1	3.4	5.3	5.6	5.1
CV%	0.9	1.1	0.9	1.4	1.5	1.3

Specificity

The Emit® II Plus Cocaine Metabolite Assay detects benzoylecgonine, the major metabolite of cocaine, in human urine.

Table 6 lists the concentrations of compounds that produce a result that is approximately equivalent to the 150 ng/mL and 300 ng/mL calibrator/control cutoffs, respectively. These concentrations are within the range of levels found in urine following use of the compound or, in the case of metabolites, the parent compound. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 7 may combine to produce a rate equal to or greater than that of the cutoff calibrator. Data presented are representative of typical performance of this assay.

Table 6 — Concentrations of Cocaine and Ecgonine that Produce a Result Approximately Equivalent to the 150 ng/mL and 300 ng/mL Benzoylecgonine Cutoffs

Compound	Concentration (µg/mL) at the 150 ng/mL Cutoff	Concentration (µg/mL) at the 300 ng/mL Cutoff
Cocaine	18–53	40–119
Ecgonine	2–6	7–20

Table 7 lists the compounds that produce a negative result by the Emit® II Plus Cocaine Metabolite Assay. Specificity testing was performed on a reference analyzer at the 150 ng/mL cutoff, which represents the greatest potential for cross-reactivity. Positive results for compounds structurally unrelated to cocaine metabolite have not been observed.

Table 7 — Concentrations of Compounds Showing a Negative Response

Compound	Concentration Tested (µg/mL) at 150 ng/mL (0.15 µg/mL) Cutoff
Acetaminophen	1000
α-Acetyl *N*, *N* dinormethadol (dinor LAAM)	25
L-α-Acetylmethadol (LAAM)	25
N-Acetylprocainamide (NAPA)	400
Acetylsalicylic Acid	1000
Amitriptyline	1000
Buprenorphine	1000
Caffeine	1000
Cimetidine	1000
Clomipramine	2.5
Clonidine	1000
Codeine	500
Cotinine	100
Cyclobenzaprine	1000
Desipramine	800
Dextromethorphan	1000
Diphenhydramine	1000
Doxepin	1000
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1000
Fluoxetine	1000
Glutethimide	500
Ibuprofen	1000
Ketamine	100
Ketorolac Tromethamine	1000
Lormetazepam	1
LSD	0.01
Meperidine	1000
Methadone	1000
Methaqualone	1500
Morphine	1000
Naproxen	1000
Nortriptyline	1000
Oxazepam	300
Phencyclidine	1000
Phenytoin	1000
Promethazine	1000
Propoxyphene	1000
Ranitidine	1000
Scopolamine	500
Secobarbital	1000
11-nor-Δ^9-THC-9-COOH	100
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2000
Zolpidem	100

Analytical Sensitivity

The sensitivity level of the Emit® II Plus Cocaine Metabolite Assay is less than 20 ng/mL. This level represents the lowest concentration of benzoylecgonine that can be distinguished from 0 ng/mL with a confidence level of 95%.

11 BIBLIOGRAPHY

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NOTES

Symbols Key

	Do not reuse
	Use By
LOT	Batch Code
REF	Catalogue Number
	Caution, consult accompanying documents
	Manufacturer
EC REP	Authorized Representative in the European Community
	Contains sufficient for <n> tests
IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation
	Consult Instructions for Use
NON STERILE	Non-sterile
CE	CE Mark
CONTENTS	Contents
	Reconstitution Volume
LEVEL	Level

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Beckman Coulter customers contact their technical assistance center.

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