LZI Cocaine Metabolite 150 Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0340 (100/37.5 mL R_1/R_2 Kit)
0341 (1000/375 mL R_1/R_2 Kit)

2ºC 8ºC

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Cocaine Metabolite 150 Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of benzoylecgonine (a cocaine metabolite) in human urine at a cutoff value of 150 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Cocaine (methylbenzoylecgonine) is an alkaloid found in the plant *Erythroxylum coca*, which is principally grown in South America. It is chemically, but not pharmacologically, related to atropine. Cocaine is a CNS stimulant; however, it also exhibits numerous undesirable side effects including cardiac toxicity and behavior responses such as paranoia and hallucinations. The most important clinical action of cocaine is its ability to block nerve conductance upon local application (3).

Cocaine sold on the street includes hydrochloride salt and crack. The salt is frequently abused by inhalation or dissolved and injected subcutaneously or intravenously. Crack is a free base form of cocaine that produces a characteristic cracking sound when burned.

Cocaine is rapidly metabolized, with less than 5 % excreted unchanged in urine. The major metabolite is benzoylecgonine. Other notable metabolites are methylecgonine and ecgonine. Cocaine metabolites are detectable in urine for 1-3 days after moderate use (4, 5). However, for long term, heavy use, the metabolites may be found in urine for up to 3 weeks (6, 7). Cocaine readily passes through a placenta into the fetus. Thus, cocaine abuse during pregnancy can adversely affect the fetal development and cause serious problems in the neonate (5).

Assay Principle

The LZI Cocaine Metabolite 150 assay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (8). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, benzoylecgonine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to the free drug; the unbound benzoylecgonine-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-benzoylecgonine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with benzoylecgonine in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and controls are sold separately and contain negative human urine with sodium azide as a preservative.*

COCAINE METABOLITE 150 Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 75 ng/mL benzoylecgonine	0342
Cutoff Calibrator: Contains 150 ng/mL benzoylecgonine	0343
Intermediate Calibrator: Contains 300 ng/mL benzoylecgonine	0344
High Calibrator: Contains 1000 ng/mL benzoylecgonine	0345

COCAINE METABOLITE 150 Controls	REF
Level 1 Control: Contains 112.5 ng/mL benzoylecgonine	0347
Level 2 Control: Contains 187.5 ng/mL benzoylecgonine	0348

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (9).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use.

Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (10). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (11). For longer storage, keep sample frozen and then thaw before use. Studies have shown benzoylecgonine analytes in urine are stable at -20ºC up to 16 months (12). Optimal storage of benzoylecgonine in urine samples occurs when frozen and stored in darkness (13). Samples should be at room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Hitachi 717 and the Synermed IR500 clinical analyzers.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 150 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: use the 112.5 ng/mL and 187.5 ng/mL for the 150 ng/mL cutoff level.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took illegal drugs and a negative test result does not necessarily mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator which contains 150 ng/mL of benzoylecgonine is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (ΔmA/min) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmA/min) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, LC/MS or (2) permitting laboratories to establish quality control procedures.
When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of benzoylecgonine in the sample may then be estimated from the calibration curve.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of benzoylecgonine. The test is not intended for quantifying this single analyte in samples.*
2. *A preliminary positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take illegal drugs.*
4. *Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
5. *Preliminary positive results should be confirmed by other affirmative, analytical chemical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human urine only.*
7. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer.

Precision:

Semi-quantitative analysis: The concentrations of the cutoff level and the two levels of controls were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	1.7	2.7	156.9 %	1.7	2.2	129.4 %
37.5 ng/mL	40.4	4.4	10.8 %	40.4	3.8	9.3 %
75 ng/mL	76.9	5.7	7.4 %	76.9	4.2	5.4 %
112.5 ng/mL	111.6	4.8	4.3 %	111.6	4.1	3.7 %
150 ng/mL	146.2	6.8	4.7 %	146.2	5.9	4.1 %
187.5 ng/mL	185.1	7.5	4.0 %	185.1	6.3	3.4 %
225 ng/mL	224.0	9.1	4.1 %	224.0	9.0	4.0 %
262.5 ng/mL	263.4	8.9	3.4 %	263.4	7.8	3.0 %
300 ng/mL	301.9	11.2	3.7 %	301.9	9.4	3.1 %

150 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
37.5 ng/mL	25 %	22	22 Neg	88	88 Neg
75 ng/mL	50 %	22	22 Neg	88	88 Neg
112.5 ng/mL	75 %	22	22 Neg	88	88 Neg
150 ng/mL	100 %	22	19 Neg/ 3 Pos	88	59 Neg/ 29 Pos
187.5 ng/mL	125 %	22	22 Pos	88	88 Pos
225 ng/mL	150 %	22	22 Pos	88	88 Pos
262.5 ng/mL	175 %	22	22 Pos	88	88 Pos
300 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: The three calibrators and two levels of controls were evaluated. Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	373.5	5.0	1.3 %	373.5	3.1	0.8 %
37.5 ng/mL	407.2	3.8	0.9 %	407.2	2.8	0.7 %
75 ng/mL	437.2	4.6	1.1 %	437.2	3.3	0.7 %
112.5 ng/mL	462.6	4.5	1.0 %	462.6	2.8	0.6 %
150 ng/mL	486.0	4.6	0.9 %	486.0	3.7	0.8 %
187.5 ng/mL	509.6	4.3	0.9 %	509.6	3.0	0.6 %
225 ng/mL	528.9	5.0	0.9 %	528.9	4.5	0.9 %
262.5 ng/mL	544.8	4.6	0.8 %	544.8	3.4	0.6 %
300 ng/mL	560.8	4.7	0.8 %	560.8	3.6	0.6 %

Qualitative analysis, continued:

150 ng/mL Cutoff Result:		Within Run (N=22)		Total Precision (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
37.5 ng/mL	25 %	22	22 Neg	88	88 Neg
75 ng/mL	50 %	22	22 Neg	88	88 Neg
112.5 ng/mL	75 %	22	22 Neg	88	88 Neg
150 ng/mL	100 %	22	19 Neg/ 3 Pos	88	73 Neg/ 15 Pos
187.5 ng/mL	125 %	22	22 Pos	88	88 Pos
225 ng/mL	150 %	22	22 Pos	88	88 Pos
262.5 ng/mL	175 %	22	22 Pos	88	88 Pos
300 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy: Eighty (80) unaltered clinical urine specimens were tested with the LZI Cocaine Metabolite 150 Enzyme Immunoassay and confirmed by either GC/MS or LC/MS. Specimens having a benzoylecgonine concentration greater than 150 ng/mL by GC/MS or LC/MS are defined as positive and specimens with concentrations below 150 ng/mL by GC/MS or LC/MS were defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

Semi-Quantitative Accuracy Study:

150 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	1*	5	31	90.0 %
Negative	20	4	15	4**	0	98.0 %

Summary of Discordant Results in Semi-Quantitative Mode:

Discrepant Sample #	Total GC/MS or LC/MS Result BE*** (ng/mL)	GC/MS or LC/MS Qualitative Result (Pos/Neg)	EIA Qualitative Result (Pos/Neg)
38*	141	-	+
41**	150	+	-
44**	167	+	-
45**	167	+	-
47**	176	+	-

Qualitative Accuracy Study:

150 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	2*	6	31	90.0 %
Negative	20	4	14	4**	0	95.0 %

Summary of Discordant Results in Qualitative Mode:

Discrepant Sample #	Total GC/MS or LC/MS Result BE*** (ng/mL)	GC/MS or LC/MS Qualitative Result (Pos/Neg)	EIA Qualitative Result (Pos/Neg)
38*	141	-	+
39*	147	-	+
41**	150	+	-
42**	152	+	-
44**	167	+	-
47**	176	+	-

*** *BE = Benzoylecgonine*

Analytical Recovery: To demonstrate linearity for sample dilution and quality control purposes (see semi-quantitative results section), a drug-free urine pool spiked with benzoylecgonine was serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

$y = 1.0488x - 0.5229$, $r^2 = 0.9992$

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
0.0	4.0	N/A
48.1	58.1	120.8 %
96.2	96.6	100.4 %
144.3	151.4	104.9 %
192.4	200.5	104.2 %
288.6	312.1	108.1 %
384.8	403.1	104.8 %
481.0	488.3	101.5 %
577.2	588.2	101.9 %
673.4	694.8	103.2 %
769.6	812.0	105.5 %
865.8	917.5	106.0 %
962.0	1017.8	105.8 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator.
The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 150 ng/mL benzoylecgonine cutoff or the maximal concentration of the compound tested that gave a response with cross-reactivity below the cutoff calibrator.

Structurally Related Cocaine Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Benzoylecgonine	150	96.03 %
Benzoylecgonine	300	102.10 %
Cocaethylene	4,000	4.58 %
Cocaine	25,000	1.5 %
Ecgonine	400,000	0.03 %
Ecgonine, Methyl Ester	500,000	0.00 %

Structurally Related Cocaine Compounds, continued:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Norcocaine	30,000	0.68 %
Atropine	500,000	0.00 %

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Acetaminophen	500,000	0.00 %
Acetylsalicylic Acid	500,000	0.00 %
Amobarbital	500,000	0.00 %
Amoxicillin	500,000	0.00 %
Amphetamine	500,000	0.00 %
Bupropion	500,000	0.00 %
Captopril	500,000	0.00 %
Caffeine	500,000	0.00 %
Chlordiazepoxide	500,000	0.00 %
Chlorpheniramine	500,000	0.00 %
Chlorpomazine	500,000	0.00 %
Codeine	500,000	0.00 %
Dextromethorphan	500,000	0.00 %
Diazepam	500,000	0.00 %
Digoxin	500,000	0.00 %
Enalapril	500,000	0.00 %
Fluoxetine	100,000	0.01 %
Glyburide	500,001	0.00 %
Ibuprofen	500,000	0.00 %
Lidocaine	500,000	0.00 %
Meperidine	500,000	0.00 %
Methadone	100,000	0.01 %
Methamphetamine	500,000	0.00 %
Methaqualone	500,000	0.00 %
Morphine	500,000	0.00 %
Nicodine	500,000	0.00 %
Nifedipine	100,000	0.00 %
Oxazepam	100,000	0.00 %
Phencyclidine	500,000	0.00 %
Phenobarbital	500,000	0.00 %
Propoxyphene	100,000	0.00 %
Ranitidine	500,000	0.00 %
Salicyluric Acid	500,000	0.00 %
Secobarbital	500,000	0.00 %
11-nor- THC-COOH	500,000	0.00 %
Valproic Acid	500,000	0.00 %
Verapamil	500,000	0.00 %

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

Interference: Endogenous Substances

The following endogenous compounds were spiked into negative urine and the two levels of controls (112.5 ng/mL and 187.5 ng/mL) for the assay. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 150 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	Qualitative Result		
		0 ng/mL (Pos/Neg)	112.5 ng/mL Control (Pos/Neg)	187.5 ng/mL Control (Pos/Neg)
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	400	Neg	Neg	Pos
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	1500	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Sodium Chloride	3000	Neg	Neg	Pos
Urea	2000	Neg	Neg	Pos

Interference: Endogenous Substances, continued:

Interfering Substances	Spiked [ ] (mg/dL)	Qualitative Result		
		0 ng/mL (Pos/Neg)	112.5 ng/mL Control (Pos/Neg)	187.5 ng/mL Control (Pos/Neg)
pH 3	N/A	Neg	Neg	Pos
pH 4	N/A	Neg	Neg	Pos
pH 5	N/A	Neg	Neg	Pos
pH 6	N/A	Neg	Neg	Pos
pH 7	N/A	Neg	Neg	Pos
pH 8	N/A	Neg	Neg	Pos
pH 9	N/A	Neg	Neg	Pos
pH 10	N/A	Neg	Neg	Pos
pH 11	N/A	Neg	Neg	Pos

Specific Gravity: Specific gravity samples ranging in value from 1.002 to 1.0275 were tested in qualitative mode against pooled processed urine samples at 0 ng/mL, 112.5 ng/mL, or 187.5 ng/mL (negative calibrator, negative control, and positive controls for the 150 ng/mL cutoff). No interference was observed.

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Additions, deletions, or changes are indicated by a change bar in the margin.

For technical assistance please call: (408) 970-8811

Manufacturer:
Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051
USA
Tel: (408) 970-8811
Fax: (408) 970-9030
www.lin-zhi.com

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