LZI Buprenorphine Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0270 (100/37.5 mL R_1/R_2 Kit)
0271 (1000/375 mL R_1/R_2 Kit)

2ºC 8ºC

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International (LZI) Buprenorphine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of norbuprenorphine (a buprenorphine metabolite) in human urine at a cutoff value of 5 ng/mL and 10 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

Buprenorphine is a semi-synthetic opioid derived from thebaine, an alkaloid of the poppy plant, *Papaver somniferum*. It is an analgesic often used as a substitution treatment for heroin addiction or opiate dependence. Buprenorphine structurally resembles morphine but has both antagonist and agonist properties (3). As an opioid partial agonist, buprenorphine can produce typical opioid effects and side effects such as euphoria and respiratory depression. However, its maximal effects are less than those of full agonists like heroin and methadone. At low doses, buprenorphine produces sufficient agonist effects to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. The agonist effects of buprenorphine increase linearly with increasing doses of the drug until it reaches a plateau and no longer continues to increase with further increases in dosage. Buprenorphine also acts as an antagonist, blocking other opioids, while allowing for some opioid effect of its own to suppress withdrawal symptoms and cravings (4). Buprenorphine is metabolized in the human liver by N-dealkylation to the pharmacologically active norbuprenorphine, which, along with the parent compound, is conjugated with glucuronic acid (5), and excreted in urine. Clearance rates are dependent on many factors, such as frequency of drug use, the amount of drug taken, metabolism rates, and even body fat content. For typical opioid-dependent patients who received a stable daily sublingual dose of 16 mg of buprenorphine and 4 mg of Naloxone for at least two weeks, 24-hour urinary elimination is approximately 11 % of daily dose (6). Therapeutically, buprenorphine is as effective as methadone but exhibits a much lower level of physical dependence. However, studies have shown that buprenorphine has abuse potential and may itself cause dependency (7).

Assay Principle

The LZI buprenorphine assay is a homogeneous enzyme immunoassay using a 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, buprenorphine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody binds to the free drug; the unbound buprenorphine-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-buprenorphine 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 buprenorphine 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.*

NORBUPRENORPHINE Calibrators/Controls	REF
Negative Calibrator	0001
Control: Contains 3 ng/mL norbuprenorphine	0272
Cutoff/Calibrator: Contains 5 ng/mL norbuprenorphine	0273
Control: Contains 7 ng/mL norbuprenorphine	0274
Cutoff/Calibrator: Contains 10 ng/mL norbuprenorphine	0275
Control: Contains 13 ng/mL norbuprenorphine	0276
Calibrator: Contains 20 ng/mL norbuprenorphine	0277
Calibrator: Contains 40 ng/mL norbuprenorphine	0278
Calibrator: Contains 75 ng/mL norbuprenorphine	0279

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagents used in the assay contain sodium azide as a preservative, which may react with lead or copper plumbing to form potentially explosive metal azide. 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, 12). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown buprenorphine analytes in urine are stable at -20ºC up to 85 days (6). Samples should be at a room temperature of 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 both samples should be forwarded 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, Beckman Coulter® AU680, 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 5 ng/mL or 10 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all six 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 each cutoff level: use the 3 ng/mL and 7 ng/mL controls for the 5 ng/mL cutoff, and use the 7 ng/mL and 13 ng/mL controls for the 10 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 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 5 ng/mL or 10 ng/mL of norbuprenorphine, 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 or 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 six calibrators. The concentration of norbuprenorphine 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 buprenorphine and/or norbuprenorphine. 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 buprenorphine and/or norbuprenorphine.*
4. *There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).*
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.*
8. *There is a possibility that metabolites of other opiate drugs may interfere with the test.*

Typical Performance Characteristics

The assay's range from 0 ng/mL to 20 ng/mL was tested in qualitative (mA/min) and semi-quantitative (ng/mL) modes using a modified NCCLS protocol. Results shown below were obtained by testing all samples in replicates of two, two runs per day for 22 days on the Hitachi 717 analyzer.

Precision:

Qualitative analysis: Typical results (mA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	400.9	3.2	0.8 %	400.9	5.1	1.3 %
2.5 ng/mL	419.6	2.7	0.6 %	419.6	4.0	0.9 %
5.0 ng/mL	439.2	3.2	0.7 %	439.2	5.0	1.1 %
7.5 ng/mL	461.2	3.3	0.7 %	461.2	4.7	1.0 %
10 ng/mL	479.2	3.2	0.7 %	479.2	4.5	0.9 %
12.5 ng/mL	495.4	3.3	0.7 %	495.4	4.8	1.0 %
15 ng/mL	511.7	3.3	0.7 %	511.7	4.6	0.9 %
17.5 ng/mL	526.8	3.2	0.6 %	526.8	4.5	0.9 %
20 ng/mL	540.3	3.5	0.6 %	540.3	4.5	0.8 %

5 ng/mL Cutoff		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
2.5 ng/mL	50 %	22	22 Neg	88	88 Neg
5 ng/mL	100 %	22	13 Neg/ 9 Pos	88	45 Neg/ 43 Pos
7.5 ng/mL	150 %	22	22 Pos	88	88 Pos
10 ng/mL	200 %	22	22 Pos	88	88 Pos

10 ng/mL Cutoff		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
2.5 ng/mL	25 %	22	22 Neg	88	88 Neg
5 ng/mL	50 %	22	22 Neg	88	88 Neg
7.5 ng/mL	75 %	22	22 Neg	88	88 Neg
10 ng/mL	100 %	22	4 Neg/ 18 Pos	88	29 Neg/ 59 Pos
12.5 ng/mL	125 %	22	22 Pos	88	88 Pos
15 ng/mL	150 %	22	22 Pos	88	88 Pos
17.5 ng/mL	175 %	22	22 Pos	88	88 Pos
20 ng/mL	200 %	22	22 Pos	88	88 Pos

Semi-quantitative analysis: 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	0.5	0.6	N/A	0.5	0.6	N/A
2.5 ng/mL	3.1	0.3	9.46 %	3.1	0.4	14.4 %
5.0 ng/mL	5.2	0.3	6.48 %	5.2	0.5	9.4 %
7.5 ng/mL	7.7	0.3	4.26 %	7.7	0.5	6.0 %
10 ng/mL	10.0	0.4	4.05 %	10.0	0.5	5.3 %
12.5 ng/mL	12.1	0.4	3.42 %	12.1	0.5	4.4 %
15 ng/mL	14.5	0.6	4.35 %	14.5	0.8	5.5 %
17.5 ng/mL	17.0	0.5	3.00 %	17.0	0.6	3.7 %
20 ng/mL	19.9	0.7	3.42 %	19.9	0.8	4.0 %

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 2 ng/mL for both the 5 ng/mL and 10 ng/mL cutoffs.

Accuracy: Ninety (90) unaltered clinical urine specimens were tested with LZI Buprenorphine Enzyme Immunoassay and confirmed with GC/MS. Specimens having a norbuprenorphine concentration greater than 5 ng/mL or 10 ng/mL by GC/MS are defined as positive, and specimens with lower concentrations by GC/MS are defined as negative in the table below. Near cutoff samples are defined as ± 50 % of the cutoff value. The correlation results are summarized as follows:

5 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	2*	7	47	96.4 %
Negative	16	6	12	0	0	100.0 %

10 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	2**	8	31	95.1 %
Negative	16	20	12	1***	0	98.0 %

The following table summarizes the results for the five discordant samples:

Cutoff Value	Assay Result	Sample Composition:	
		NBUP (GC/MS)	BUP (GC/MS)
5 ng/mL	Positive*	4.1 ng/mL	0.0 ng/mL
5 ng/mL	Positive*	4.4 ng/mL	0.0 ng/mL
10 ng/mL	Positive**	8.5 ng/mL	3.2 ng/mL
10 ng/mL	Positive**	9.5 ng/mL	0.0 ng/mL
10 ng/mL	Negative***	10.2 ng/mL	0.0 ng/mL

Linearity: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free urine pool spiked with pure norbuprenorphine 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:

$y = 1.0026x + 0.9053$, $r^2 = 0.991$

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
2	2.3	115.0 %
5	5.0	99.6 %
10	9.9	99.4 %
20	20.7	103.7 %
30	33.2	110.8 %
40	44.4	111.1 %
50	52.7	105.3 %
60	56.3	93.8 %
70	71.4	102.1 %

Specificity: Cross-reactivity of various potentially interfering drugs was tested by spiking a final concentration of 100,000 ng/mL of each substance into drug-free urine, and then evaluated with the assay's calibrated dose-response curve. Cross-reactivity of the parent drug buprenorphine and its glucuronic acid metabolite is listed below as well. The assay detects the parent drug buprenorphine equally to norbuprenorphine, but has only minimal cross reactivity to either of the glucuronides.
The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 5 ng/mL and 10 ng/mL norbuprenorphine cutoff. For compounds tested at 100,000 ng/mL and which gave an immunoassay result below 2 ng/mL, which is the assay's limit of detection, equivalent concentration and percent cross reactivity are listed as "not detected" ("ND").

Structurally Related Buprenorphine Compounds:

Compound	Equivalent [ ] to 5 ng/mL	Equivalent [ ] to 10 ng/mL	% Cross-Reactivity
Buprenorphine	4.9	9.9	101 %
Buprenorphine-Glucuronide	3846	7692	0.1 %
Norbuprenorphine-Glucuronide	556	1111	0.9 %

Structurally Related Opiate Compounds*:

Compound	Equivalent [ ] to 5 ng/mL	Equivalent [ ] to 10 ng/mL	% Cross-Reactivity
6-Acetylcodeine	ND	ND	ND
Codeine	ND	ND	ND
Dextromethorphan	ND	ND	ND
Dihydrocodeine	ND	ND	ND
Heroin	192,308	384,615	0.003 %
Hydrocodone	ND	ND	ND
Hydromorphone	ND	ND	ND
Levorphanol	70,423	140,845	0.007 %
6-Monoacetylmorphine	ND	ND	ND
Morphine	ND	ND	ND
Morphine-3-Glucuronide	ND	ND	ND
Morphine-6-Glucuronide	ND	ND	ND
Nalorphine	ND	ND	ND
Naloxone	ND	ND	ND
Naltrexone	ND	ND	ND
Norcodeine	ND	ND	ND
Noroxycodone HCl	ND	ND	ND
Noroxymorphone HCl	ND	ND	ND
Oxycodone	ND	ND	ND
Oxymorphone	ND	ND	ND

**There is a possibility that metabolites of the compounds listed above may interfere with buprenorphine immunoassays and cause false results.*

Structurally Unrelated Pharmacological Compounds:**

Compound	Equivalent [ ] to 5 ng/mL	Equivalent [ ] to 10 ng/mL	% Cross-Reactivity
α-Methadol	ND	ND	ND
Citalopram	ND	ND	ND
EDDP	ND	ND	ND
EMDP	185,185	370,370	0.003 %
Fluoxetine	ND	ND	ND
Gabapentin	ND	ND	ND
Imipramine	ND	ND	ND
LAAM	ND	ND	ND
Meperidine	ND	ND	ND
Methadone	ND	ND	ND
Norpropoxyphene	ND	ND	ND
Paroxetine	ND	ND	ND
Sertraline	ND	ND	ND
Tramadol	ND	ND	ND

*** It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedure errors.*

Interference: Endogenous Substances

The following endogenous compounds were spiked into negative urine and the three levels of controls (3 ng/mL, 7 ng/mL, and 13 ng/mL) for the assay. The spiked solution was evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiologically relevant concentrations as all spiked samples gave correct responding preliminary positive/negative results against the cutoff value of 5 ng/mL or 10 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	3 ng/mL Control (ng/mL)	7 ng/mL Control (ng/mL)	13 ng/mL Control (ng/mL)
Acetone	1000	0.0	3.2	6.5	12.2
Ascorbic Acid	400	0.4	3.3	6.5	13.2
Creatinine	500	0.9	3.8	7.1	13.0
Galactose	10	0.4	3.5	6.4	11.7
γ-Globulin	500	0.0	3.0	8.1	11.2
Glucose	1500	0.0	3.1	7.1	11.5
Hemoglobin	300	0.4	4.0	8.2	13.0
NaCl	6000	0.9	3.9	7.8	13.0
Oxalic Acid	100	0.5	3.3	6.7	11.8
Human Serum Albumin	500	0.0	3.2	7.1	11.8
Riboflavin	7.5	0.0	3.0	7.9	12.5
Urea	2000	0.0	3.3	7.2	11.6
Ethanol	1000	0.0	3.0	8.1	11.9
pH 3	N/A	0.0	3.3	5.9	13.6
pH 11	N/A	0.0	3.7	8.0	12.7

Specific Gravity: Samples ranging in specific gravity from 1.001 to 1.027 were tested with the assay in the presence of 0 ng/mL, 3 ng/mL, 7 ng/mL, and 13 ng/mL of norbuprenorphine, and no interference was observed.

Note: All endogenous substances listed above, including specific gravity, were also tested in qualitative mode. No interference is observed. The results were identical to the semi-quantitative mode as all samples gave correct positive/negative results corresponding to the cutoff value of 5 ng/mL or 10 ng/mL.

Bibliography:

1. Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph **73** (1986).
2. Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **53**(69): 11970 (1988).
3. Baselt, R.C., Disposition of toxic drugs and chemicals in man. 5th edition. Chemical Toxicology Institute, Foster City, CA, 103-105 (2000).
4. Center for Substance Abuse Treatment. Clinical Guidelines for the Use of Buprenorphine in the Treatment of Opioid Addiction. Treatment Improvement Protocol (TIP) Series 40. DHHS Publication No. (SMA) 04-3939. Rockville, Md: Substance Abuse and Mental Health Services Administration, (2004).
5. Cone, E.J., Gorodetzky, D., Yousefnejad, W.F., Buchwald, W.F. and Johnson, R.E., The metabolism and excretion of buprenorphine in humans. *Drug Metab Dispos* **12**: 577-581, (1984).
6. McCance-Katz, et. al., The In Vivo Glucuronidation of Buprenorphine and Norbuprenorphine Determined by Liquid Chromotography-Electrospray Ionization-Tandem Mass Spectrometry, *Therapeutic Drug Monitoring*, **28**(2):245-251 (2006).
7. Yokell, M.A., et al., Buprenorphine and buprenorphine/naloxone diversion, misuse, and illicit use: an international review, *Current drug abuse reviews*, **4**:28 (2011).
8. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**: 846 (1972).
9. Sodium Azide. National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html
10. Yahya, A.M., McElnay, J.C., and P.F. D'Arcy. Drug absorption to glass and plastics. *Drug Metabol Drug Interact*. **6**(1):1-45 (1988).
11. Dixon, R. B., Stability study of opioids and benzodiazepines in urine samples by liquid chromatography tandem mass spectrometry, Journal of Analytical Science and Technology, 6:17 (2015).
12. Gonzales, E., et al., Stability of pain-related medications, metabolites, and illicit substances in urine, Clinica Chimica Acta, 416:80-85 (2013).

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

Authorized European Rep. within the EU:
CEpartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands
www.cepartner4u.eu

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