LZI Tramadol Enzyme Immunoassay

REF 0410 (100/37.5 mL R_1/R_2 Kit)
0411 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

IVD For In Vitro Diagnostic Use Only

Lin-Zhi International, Inc.

Intended Use

The LZI Tramadol Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of tramadol in human urine at a cutoff value of 100 ng/mL when calibrated against tramadol. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.
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 gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) or (2) permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical result. A more specific alternative chemical confirmatory method (e.g., gas or liquid chromatography and mass spectrometry) must be used to obtain a confirmed analytical result (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

Tramadol is a synthetic analog of codeine, and is an opioid analgesic used in treating moderate to moderately severe pain. It has a wide range of applications, including treatment for restless leg syndrome, acid reflux, and fibromyalgia.
Tramadol is available in various pharmaceutical forms, particularly in solution for intravenous, intramuscular or subcutaneous injection. It can be administered via an immediate release or sustained release formulation (3).
The opioid agonistic effect of tramadol and its major metabolites are almost exclusively mediated by its actions at the μ-opioid receptor. In addition to its opioid actions, tramadol inhibits the neuronal reuptake of norepinephrine and serotonin (3).
Tramadol is a prodrug which is converted to O-desmethyltramadol. Tramadol is mainly metabolized by *O*- and *N*-demethylation and by conjugation reactions forming glucuronides and sulfates (4-6). Tramadol and its metabolites are mainly excreted via the kidneys. The mean elimination half-life is about six hours (7-12).

Assay Principle

The LZI Tramadol Enzyme Immunoassay 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 (13). The drug-labeled G6PDH conjugate is traceable to a commercially available tramadol standard and referred to as tramadol-labeled G6PDH conjugate. Enzyme activity decreases upon binding to the antibody, and the tramadol concentration in the sample is measured in terms of enzyme activity. In the absence of tramadol in the sample, tramadol-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when tramadol is present in the sample, antibody would bind to free tramadol; the unbound tramadol-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 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a mouse monoclonal anti-tramadol 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 tramadol-labeled glucose-6-phosphate dehydrogenase (G6PDH) 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.*

TRAMADOL Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 50 ng/mL tramadol	0412
Cutoff Calibrator: Contains 100 ng/mL tramadol	0413
Intermediate Calibrator: Contains 225 ng/mL tramadol	0414
High Calibrator: Contains 400 ng/mL tramadol	0415

TRAMADOL Controls	REF
Level 1 Control: Contains 75 ng/mL tramadol	0417
Level 2 Control: Contains 125 ng/mL tramadol	0418

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 (14).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: 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

Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to one month. For longer storage, keep sample frozen at -20ºC and then thaw before use (15).
Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to a laboratory for testing.
Handle all urine specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm 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 Beckman Coulter AU480 automated clinical analyzer.

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 12 μL sample, 120 μL of antibody reagent (R_1), 45 μL of enzyme conjugate reagent (R_2), 10 μL dilution following addition of R_2 in 37ºC incubation temperature, 14-18 reading frame, FIXED method, and 340 nm primary wavelength.
For qualitative analysis, use the 100 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 75 and 125 ng/mL.

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 positive test result does not necessarily mean a person took a specific drug and a negative test result does not necessarily mean a person did not take a specific drug. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 100 ng/mL of tramadol, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (ΔmAU) equal to or greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance (ΔmAU) 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 tramadol in the sample may then be estimated from the calibration curve.

Limitations

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

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU480 automated chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

100 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Tramadol Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0 %	22	22 Neg	88	88 Neg
25 ng/mL	25 %	22	22 Neg	88	88 Neg
50 ng/mL	50 %	22	22 Neg	88	88 Neg
75 ng/mL	75 %	22	22 Neg	88	88 Neg
100 ng/mL	100 %	22	6 Neg/ 16 Pos	88	35 Neg/ 53 Pos
125 ng/mL	125 %	22	22 Pos	88	88 Pos
150 ng/mL	150 %	22	22 Pos	88	88 Pos
175 ng/mL	175 %	22	22 Pos	88	88 Pos
200 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

100 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Tramadol Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0 %	22	22 Neg	88	88 Neg
25 ng/mL	25 %	22	22 Neg	88	88 Neg
50 ng/mL	50 %	22	22 Neg	88	88 Neg
75 ng/mL	75 %	22	22 Neg	88	88 Neg
100 ng/mL	100 %	22	7 Neg/ 15 Pos	88	40 Neg/ 48 Pos
125 ng/mL	125 %	22	22 Pos	88	88 Pos
150 ng/mL	150 %	22	22 Pos	88	88 Pos
175 ng/mL	175 %	22	22 Pos	88	88 Pos
200 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy: Eighty-six (86) unaltered clinical urine specimens were tested with the LZI Tramadol Enzyme Immunoassay and confirmed with LC/MS. Specimens with a tramadol concentration greater than or equal to 100 ng/mL by LC/MS are defined as positive, and specimens with a tramadol concentration below 100 ng/mL by LC/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:

Semi-Quantitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	0	1*	9	33	97.7 %
Negative	20	7	15	1**	0	97.7 %

The following table summarizes the results for the semi-quantitative discordant samples:

Sample #	Tramadol LC/MS (ng/mL)	Pos/Neg Result	AU480 EIA Semi-Quantitative Result (ng/mL)	Pos/Neg Result
38*	71	-	221.6	+
46**	114	+	80.9	-

Qualitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	0	2*	9	33	97.7 %
Negative	20	7	14	1**	0	95.3 %

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

Sample #	Tramadol LC/MS (ng/mL)	Pos/ Neg Result	O-desmethyl Tramadol LC/MS (ng/mL)	AU480 EIA Qualitative Result (mAU)	Qualitative Cutoff Rate (mAU)	Pos/ Neg Result
38*	71	-	118	375.5	192.3	+
42*	85	-	56	198.3	192.3	+
46**	114	+	57	137.5	174.6	-

Analytical Recovery: To demonstrate recovery for purposes of sample dilution and quality control of the entire assay range, a drug free–urine pool spiked with tramadol at 400 ng/mL was serially diluted. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value.

Target Concentration (ng/mL)	Determined Concentration Range (ng/mL)	Determined Concentration Average (ng/mL)	Average % Recovery
400	395.2 – 433.3	412.7	103.2 %
360	350.3 – 404.5	377.5	104.9 %
320	316.5 – 371.6	346.9	108.4 %
280	277.6 – 313.0	297.8	106.4 %
240	221.7 – 279.7	245.1	102.1 %
200	202.0 – 215.5	210.0	105.0 %
160	168.2 – 180.2	174.5	109.1 %
120	122.8 – 134.2	130.1	108.4 %
80	78.0 – 84.6	80.9	101.1 %
40	35.6 – 43.0	39.3	98.2 %
0	-4.6 – 1.3	-2.2	N/A

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into a drug free–urine pool to various concentrations and evaluated with the assay's calibration curve in both qualitative and semi-quantitative modes. The following table lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative). Compounds tested at high concentration (100,000 ng/mL) with results below the cutoff value were listed as Not Detected (ND). Compounds tested below the high concentration (100,000 ng/mL) that gave a result below the cutoff value were given a "< %" value.

Tramadol and Metabolites:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Tramadol	100	100.00 %
O-desmethyl cis-tramadol HCl	166	60.24 %
N-desmethyl-cis-tramadol	10,000	1.00 %
N, O-didesmethyl tramadol	15,000	0.67 %
O-desmethyl tramadol β-D-glucuronide	90	111.11 %

Structurally Related Compounds:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Ketamine	100,000	ND
Dehydronorketamine	10,000	< 1.00 %
Norketamine	20,000	< 0.50 %
Phencyclidine	100,000	ND
Venlafaxine	100,000	ND
O-desmethylvenlafaxine	100,000	ND

Structurally Unrelated Compounds:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Tramadol Concentration		
		0 ng/mL	75 ng/mL Control	125 ng/mL Control
6-Acetylmorphine	100,000	ND	Neg	Pos
Acetaminophen	100,000	ND	Neg	Pos
Acetylsalicylic Acid	100,000	ND	Neg	Pos
Aliemazine Tartrate	100,000	ND	Neg	Pos
Amitriptyline	100,000	ND	Neg	Pos
Amlodipine Besylate	100,000	ND	Neg	Pos
d-Amphetamine	100,000	ND	Neg	Pos
Amoxicillin	100,000	ND	Neg	Pos
Atorvastatin	100,000	ND	Neg	Pos
Benzoylecgonine	100,000	ND	Neg	Pos
Buprenorphine	100,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
Desipramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Doxylamine Succinate	100,000	ND	Neg	Pos
Duloxetine	100,000	ND	Neg	Pos
Etavirenz	100,000	ND	Neg	Pos
Fentanyl (citrate)	100,000	ND	Neg	Pos
Fluoxetine	100,000	ND	Neg	Pos
Fluphenazine	100,000	ND	Neg	Pos
Gabapentin	100,000	ND	Neg	Pos
Hydrocodone	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Neg	Pos
Hydroxyzine Pamoate	100,000	ND	Neg	Pos
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
JWH-073 (SPICE I)	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos
Loratadine	100,000	ND	Neg	Pos
Lorazepam	100,000	ND	Neg	Pos
Losartan	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100,000	ND	Neg	Pos
Meperidine	100,000	ND	Neg	Pos
Metformin	100,000	ND	Neg	Pos
Methadone	100,000	ND	Neg	Pos
d-Methamphetamine	100,000	ND	Neg	Pos
Methapyrilene HCl	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
Morphine	100,000	ND	Neg	Pos
Nicotine	100,000	ND	Neg	Pos
Niflumic Acid	100,000	ND	Neg	Pos
Nortriptyline	100,000	ND	Neg	Pos
Omeprazole	100,000	ND	Neg	Pos
Oxazepam	100,000	ND	Neg	Pos
Oxycodone	100,000	ND	Neg	Pos
Oxymorphone	100,000	ND	Neg	Pos
d-Propoxyphene	100,000	ND	Neg	Pos
(1S,2S)-(+)Pseudoephedrine	100,000	ND	Neg	Pos
Quetiapine	100,000	ND	Neg	Pos
Ranitidine	100,000	ND	Neg	Pos
Salbutamol (Albuterol)	100,000	ND	Neg	Pos
Sertraline	100,000	ND	Neg	Pos
THC-COOH (11-Nor-Δ-9-THC-9-carboxylic acid)	100,000	ND	Neg	Pos
l-Thyroxine	100,000	ND	Neg	Pos
Trioridazine	100,000	ND	Neg	Pos
(+)Verapamil HCl	100,000	ND	Neg	Pos
Zolpidem	1,250	<8.00 %	Neg	Pos
Zolpidem phenyl-4-carboxylic acid	10,000	<1.00 %	Neg	Pos
Zolpidem-6-carboxylic acid	10,000	<1.00 %	Neg	Pos

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

Endogenous and Preservatives Compound Interference Study:
Various potentially interfering endogenous and preservative substances were tested for interference with the assay. Test compounds were split into three portions each and either left un-spiked or spiked to a tramadol concentration of either 75 or 125 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. Only the preservative Boric Acid (1 % w/v) was found to cause interference with the assay.

Endogenous and Preservatives Compound Interference Study, continued:

Endogenous or Preservative Substance	Spiked [ ] (mg/dL)	Spiked Tramadol Concentration		
		0 ng/mL	75 ng/mL Control	125 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride (CaCl2)	300	Neg	Neg	Pos
Ciprofloxacin	1	Neg	Neg	Pos
Citric Acid (pH 3)	800	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	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos
Riboflavin	0.3	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	6000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos

pH Interference Study: pH 3 to pH 11 was tested for interference with the assay. Each pH level was split into three portions each and either left un-spiked or spiked to a tramadol concentration of either 75 or 125 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No pH interference was observed.

pH	Spiked Tramadol Concentration		
	0 ng/mL	75 ng/mL Control	125 ng/mL Control
pH 3	Neg	Neg	Pos
pH 4	Neg	Neg	Pos
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Specific Gravity: Samples ranging in specific gravity from 1.000 to 1.030 were split into three portions each and either left un-spiked or spiked to a tramadol concentration of either 75 or 125 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No interference was observed.

Symbols Used

EC REP	Authorized Representative		Manufacturer
	Biological Risks	REAGENT 1	R_1, Antibody/ Substrate Reagent
CE	CE Mark	REAGENT 2	R_2, Enzyme-Drug Conjugate Reagent
	Consult Instructions for Use	REF	Reference Number
CONTENTS	Contents	SDS	Safety Data Sheet
GTIN	Global Trade Item Number	8°C 2°C	Temperature Limits
IVD	*In Vitro* Diagnostic medical device	T.K.	Test Kit Number
LOT	Lot Number		Use-by Date

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

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