LZI Ecstasy Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0160 (100/37.5 mL R_1/R_2 Kit)
0161 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Ecstasy Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of MDMA and related compounds in human urine at a cutoff value of 500 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

Ecstasy drugs are a group of amphetamine derivatives, including MDMA (3,4-methylenedioxymethamphetamine), MDA (3,4-methylenedixoy-amphetamine), and MDEA (3,4-methylenedioxyethylamphetamine). They are central nervous system (CNS) stimulants. At a light dose, ecstasy drugs produce euphoria and an increase in self-awareness. However, they are popularly abused for their psychotropic effects at high doses and become hallucinogenic with a loss of behavioral control. Toxic overdose causes depression, uncontrolled body fluid excretion, cardiac arrhythmias, and sleep disorders. Since there is no known medical application of ecstasy drugs, and high abuse potential, the US DEA lists both MDMA and MDA as schedule I drugs. After ingestion of the drug, MDMA is known to metabolize to MDA by demethylation. Within the human body, most of the drug is eliminated through urinary excretion. Most of the urinary excretion is unchanged MDMA with a small fraction of MDA. Other urinary excretions include mono- and dihydroxy-derivatives that appear as glucuronide conjugates (3). Detection of MDMA or its metabolites in urine indicates use of ecstasy.

Assay Principle

The LZI Ecstasy 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 (4). 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, ecstasy-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 ecstasy-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotin-amide 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-ecstasy 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 ecstasy 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.*

ECSTASY (MDMA) Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 100 ng/mL ecstasy	0162
Cutoff Calibrator: Contains 500 ng/mL ecstasy	0163
Intermediate Calibrator: Contains 750 ng/mL ecstasy	0164
High Calibrator: Contains 1000 ng/mL ecstasy	0165

ECSTASY (MDMA) Controls	REF
Level 1 Control: Contains 375 ng/mL ecstasy	0167
Level 2 Control: Contains 625 ng/mL ecstasy	0168

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 (5).*
- *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 (6). 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 (7, 8). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown MDMA analytes in urine are stable at -20ºC up to 24 months (8, 9). 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 analyzer 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 500 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: 375 ng/mL and 625 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 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 500 ng/mL of ecstasy 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 ecstasy 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 ecstasy. 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 chemistry 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:

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

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	254.4	2.3	0.90 %	252.4	2.4	0.9 %
375 ng/mL	327.9	2.5	0.76 %	326.9	2.6	0.8 %
500 ng/mL	384.2	3.1	0.81 %	383.2	1.2	0.3 %
625 ng/mL	420.8	3.5	0.84 %	420.3	3.3	0.8 %
1000 ng/mL	453.5	3.3	0.72 %	453.6	3.4	0.8 %

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

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
375 ng/mL	381.4	5.2	1.4 %	367.6	8.7	2.4 %
500 ng/mL	517.4	8.1	1.6 %	502.6	9.5	1.3 %
625 ng/mL	649.0	11.4	1.8 %	637.1	8.2	1.3 %

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 50 ng/mL and is supported by the recovery study (see Analytical Recovery section below).

Accuracy: One hundred and twenty seven (127) clinical urine specimens were tested with the LZI Ecstasy EIA, 96 samples were found positive, and 28 samples were found negative. All positive samples were confirmed with GC/MS. All three discrepant samples are border-line negative.

500 ng/mL Cutoff		GC/MS		% Agree-ment
		Positive	Negative	
LZI MDMA EIA	Positive	96	3*	94 %
	Negative	0	28	100 %

** All three discordant samples were flagged as border-line negative by GC/MS.*

In addition to the above study, 25 samples were diluted in order to obtain near the cutoff value. The following table showed the results of diluted clinical sample analysis:

Number of Samples	Concentration of Samples (ng/mL)	Observed Result
7	508-610	Positive
7	428-480	Positive
10	302-461	Negative
1	450	Positive

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), drug-free urine pool spiked with ecstasy was serially diluted. Each sample was run in five 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 follow:

$y = 1.0047x - 3.2922$, $r^2 = 0.9983$

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery	% CV
50	55.0	110.0	8.1 %
125	114.3	91.5	1.5 %
230	215.4	93.6	1.6 %
370	378.2	102.2	1.0 %
450	458.9	102.0	2.3 %
550	547.4	99.5	5.7 %
650	644.1	99.1	3.6 %
780	767.2	98.4	6.2 %
920	904.1	98.3	4.1 %

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 table below 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).

Structurally Related Ecstasy Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
MDMA	500	Positive
MDEA	500	Positive
MDA	1,000	Positive
d,l-BDB*	1,200	Positive
PMMA*	1,400	Positive
MBDB*	2,000	Positive
PMA*	4,000	Positive
HMMA*	100,000	Negative

** **BDB**:3,4-Methylenedioxyphenyl-2-butanamine; **PMMA**:p-Methoxymethamphetamine; **PMA**: p-Methoxyamphetamine; **MBDB**: N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; **HMMA**: 4-Hydroxy-3-methoxymethamphetamine.*

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Acetaminophen	1000	Negative
Acetylsalicylic Acid	1000	Negative
Amitriptyline	1000	Negative
Amobarbital	1000	Negative
d-Amphetamine	1000	Negative
l-Amphetamine	100	Negative
Benzoylecgonine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine	1000	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Ephedrine	1000	Negative
Imipramine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1000	Negative
Methadone	1000	Negative
d-Methamphetamine	800	Negative
l-Methamphetamine	50	Negative
Methaqualone	1000	Negative
Morphine	1000	Negative
Nortriptyline	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Phentermine	300	Negative
d,l-Phenylpropanolamine	1000	Negative
Promethazine	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Valproic Acid	1000	Negative

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

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