LZI Oral Fluid Ecstasy (MDMA) Enzyme Immunoassay

REF S0160 (75/37.5 mL R_1/R_2 Kit)
S0161 (750/375 mL R_1/R_2 Kit)

2ºC 8ºC

FOR RESEARCH & DEVELOPMENT USE ONLY

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Ecstasy Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of ecstasy (MDMA) and related compounds in neat human oral fluid, collected into an LZI Oral Fluid Collector, at the cutoff value of 25 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. This is a Non-FDA Approved assay for Research & Development use only and as such should not be repackaged for in vitro diagnostic use.

The assay provides a rapid screening procedure for determining the presence of ecstasy in human oral fluid. 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) is the preferred confirmatory method (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-methylenedixoyamphetamine), and MDEA (3,4-methylenedioxyethylamphetamine). They are central nervous system (CNS) stimulants. At light dose, ecstasy drugs produce euphoria and increase self-awareness. However, they are popularly abused for their psychotropic effects and at high dosages become hallucinogenic and cause loss of control of behavior. Toxic overdose causes depression, uncontrolled body fluid excretion, cardiac arrhythmias, and sleep disorder. Since there is no known medical application of ecstasy drugs with high abuse potential, the US DEA lists both MDMA and MDA as schedule I drugs. After ingestion of the drug, MDMA is known to be metabolized into MDA by demethylation. The majority of MDMA is excreted unchanged into the urine, while a small amount is introduced into the oral fluid through passive diffusion of the drug from the blood stream. Detection of MDMA or its metabolites in saliva indicates use of ecstasy (3).

Assay Principle

The LZI Oral Fluid Ecstasy Enzyme Immunoassay is a homogeneous enzyme immunoassay (4) with ready-to-use liquid reagents. 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. 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 free drug and the unbound ecstasy-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 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 ecstasy-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 synthetic oral fluid matrix with sodium azide as a preservative.*

ORAL FLUID ECSTASY Calibrator/Control	REF #
Oral Fluid Negative Calibrator	S0001
Cutoff Calibrator: Contains 25 ng/mL ecstasy	S0163
High Calibrator: Contains 50 ng/mL ecstasy	S0165
Low Calibrator / Level 1 Control: Contains 15 ng/mL ecstasy	S0167
Intermediate Calibrator / Level 2 Control: Contains 35 ng/mL ecstasy	S0168

Collectors**

*** Collectors are sold separately.*

ORAL FLUID Collector	REF #
LZI Oral Fluid Collector: 50 mL Polypropylene Centrifuge Tube	S0000b

Precautions and Warning

- *This test is a Non-FDA approved assay and is for Research & Development use only. This test should not be repackaged for in vitro diagnostic use.*
- *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 (8/16/76).*
- *Do not use the reagents beyond their expiration dates.*

Reagent Preparation and Storage

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

Specimen Storage and Shipping

Note: If oral fluid samples cannot be analyzed immediately, they may be stored in amber glass vials and refrigerated (2-8ºC) for up to seven days or frozen (-20ºC) for up to two months (8).
Samples should always be shipped cold (2-8ºC), packed in gel ice, and shipped for next day delivery (within 24 hours). Failure to store or ship samples under these conditions may result in a significant decrease in recovery of analyte. Please see additional details in the Specimen Collection and Handling section below.

Specimen Collection and Handling

Oral fluid samples should be collected into a device without an absorbing pad, such as the LZI Oral Fluid Collector (a 50 mL polypropylene centrifuge tube) (7).
Prior to testing, samples should be frozen overnight (at minimum) and then allowed to thaw at room temperature. Samples should then be spun for five minutes at 3000 rpm to remove particulates. Only the clear top layer should be assayed for EIA testing and/or confirmatory testing. Samples should be at room temperature (18-25ºC) for testing.
Samples do not require dilution or any additional correction factors.
Fresh and properly stored oral fluid samples should be within the normal pH range of 6-8; however, any sample with pH ranging from 3-10 can be tested without any pretreatment of the samples.
Handle all oral fluid 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 Hitachi 717. If other instruments are used, performance will need to be validated by the laboratory.

Assay Procedure

Analyzers with specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. Typical assay parameters used for the Hitachi 717 analyzer include a 48 µL sample, 150 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) in 37ºC incubation temperature, 30-35 reading frames, and 340 nm primary wavelength.
For qualitative analysis, use the 25 ng/mL cutoff calibrator. For semi-quantitative analysis, use all five calibrators and controls. 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: 15 ng/mL and 35 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, guidelines, and regulations.

Results

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

Qualitative: The cutoff calibrator, which contains 25 ng/mL of ecstasy, is used as a reference for distinguishing 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 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.
This mode requires a calibration curve that can be established with the five assay calibrators and controls.

Limitations

1. *A 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 positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take ecstasy.*
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, endogeneous or exogeneous interferents).*
5. *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 oral fluid only.*

Typical Performance Characteristics

The results shown below were obtained with a Hitachi 717 analyzer.

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free oral fluid 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 concentration of the non-ecstasy compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Structurally Related Ecstasy Compounds:

Cross-reactant	Concentration (ng/mL)	Cross-reactivity
MDMA	25	Positive
MDEA	25	Positive
MDA	40	Positive
PMMA	50	Positive
PMA	50	Positive

Structurally Unrelated Pharmacological Compounds:

Cross-reactant	Concentration (µg/mL)	Cross-reactivity
Acetaminophen	100	Negative
Acetylsalicyclic acid	250	Negative
Amorbarbital	100	Negative
d-Amphetamine	15	Negative
l-Amphetamine	10	Negative
Benzoylecognine	100	Negative
Benzphetamine	200	Negative
Bromopheniramine	200	Negative
Bupropion	125	Negative
Buspirone	250	Negative
Caffeine	300	Negative
Chlorpheniramine	1	Negative
Codeine	250	Negative
Cocaine	60	Negative
Chlorpromazine	200	Negative
Dextromethorphen	2.5	Negative
Doxepine	25	Negative
d-Ephedrine	300	Negative
d,l-Ephedrine	90	Negative
l-Ephedrine	60	Negative
3-OH Tyramine	250	Negative
Isoxsuprine	250	Negative
Meperidine	150	Negative
Mephentermine	12	Negative
l-Methamphetamine	1	Negative
d-Methamphetamine	15	Negative
Morphine	100	Negative
Oxazepam	200	Negative
Phencyclidine	25	Negative
Phenethylamine	5	Negative
Phenmetrazine	15	Negative

Structurally Unrelated Pharmacological Compounds, continued:

Cross-reactant	Concentration (µg/mL)	Cross-reactivity
Phenobarbital	200	Negative
Phentermine	2.5	Negative
d-Phenylpropanolamine	250	Negative
d,l-Phenylpropanolamine	50	Negative
l-Phenylpropanolamine	25	Negative
Procainamide	125	Negative
Promethazine	100	Negative
Propoxyphene	100	Negative
Propranolol	300	Negative
d-Pseudoephedrine	25	Negative
l-Pseudoephedrine	250	Negative
Ranitidine	10	Negative
Secobarbital	200	Negative
Trazodone	50	Negative
Trifluoperazine	250	Negative
Tyramine	50	Negative
Valproic Acid	300	Negative

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

Bibliography:

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