LZI Oral Fluid Cannabinoid Enzyme Immunoassay

REF S0070 (75/37.5 mL R_1/R_2 Kit)
S0071 (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 Cannabinoid Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of cannabinoids in neat human oral fluid, collected into a Salivette collector (manufactured by Sarstedt), at a cutoff value of 4 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 cannabinoids 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

The principal, active constituent in marijuana (or hashish), obtained from the Cannabis sativa plant, is Δ^1-3, 4-trans tetrahydrocannabinol, frequently referred to as Δ^9-tetrahydocannabinol or Δ^9-THC. Cannabis has been used for its euphoric effects for over 4000 years (3). It is one of the most commonly used illicit drugs in the United States.

Marijuana is frequently self-administered for its mood-altering properties. Chronic use has been shown to cause reversible psychological impairment, an abstinence syndrome, and tolerance development (4). At low doses, it produces mixed depressant and stimulant effects; at higher doses, it acts as a CNS depressant (5-7).

Δ^9-THC is easily absorbed by inhalation (smoking) or ingestion through the gastrointestinal tract. Due to its highly fat-soluble nature, Δ^9-THC is readily deposited in fatty tissues, where it may remain for days or even weeks (5). It is primarily metabolized in the liver and produces a variety of compounds, the primary one being 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (11-nor-Δ^9-THC-9-COOH) (6, 7). Approximately 70% of THC is excreted in feces and urine within 72 hours of administration. However, the THC-acid metabolite is rarely ever detected in the oral fluid of a marijuana smoker (8, 9).

Assay Principle

The LZI Oral Fluid Cannabinoid Enzyme Immunoassay is a homogeneous enzyme immunoassay 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 (10). 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, Δ^9-THC-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 will bind to free drug, and the unbound Δ^9-THC-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- Δ^9-THC 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 Δ^9-THC-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 a negative synthetic oral fluid matrix with sodium azide as a preservative.*

ORAL FLUID CANNABINOID Calibrator/Control	REF #
Oral Fluid Cannabinoid Negative Calibrator	S0072
Cutoff Calibrator: Contains 4 ng/mL Δ^9-THC	S0073
High Calibrator: Contains 20 ng/mL Δ^9-THC	S0075
Low Calibrator / Level 1 Control: Contains 2 ng/mL Δ^9-THC	S0077
Intermediate Calibrator / Level 2 Control: Contains 8 ng/mL Δ^9-THC	S0078

Buffers and Collection Kits**

***Buffers and Collection Kits are sold separately.*

ORAL FLUID Buffers & Collection Kits	REF #
Oral Fluid Recovery Buffer, 1000mL	S0002
Oral Fluid 100 Collector Kit	S0005
Oral Fluid 1000 Collector Kit	S0006

Precautions and Warnings

- *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 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 21 days or frozen (-20ºC) for up to 21 days. Studies have been performed up to 21 days to show cannabinoids are stable in oral fluid. No further study was conducted beyond 21 days.

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.

Specimen Collection and Handling

For accurate sample analysis, the oral fluid should be centrifuged and then swabs should be soaked in an equal volume of the LZI Oral Fluid Recovery Buffer before a second centrifugation. For accurate sample analysis, the values of the cutoff and the semi-quantitative determination should be adjusted by a dilution factor of 1.67.

Oral fluid samples should be collected using the Sarstedt Salivette oral fluid collection device. Refer to LZI's Oral Fluid Sample Preparation sheet for instructions (11, 12).

Fresh and properly stored oral fluid samples should be within the normal pH range of 6-8; however, any sample with pH ranging from 1-13 can be tested without any pretreatment of the samples.

Samples collected into the Sarstedt Salivette oral fluid collection device require dilution. Concentrations determined by this collection method require an additional correction factor multiplication of 1.67.

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 enzyme 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 the above indicated specifications 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 60 µL sample, 135 µL of antibody reagent (R_1), 68 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 4 ng/mL cutoff calibrator. For semi-quantitative analysis, use all five calibrators and controls. 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: 2 ng/mL and 8 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 a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 4 ng/mL of Δ^9-THC, 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 cannabinoids (THC). The test is not intended for quantifying these single analytes 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 cannabinoids (THC).*
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 the 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-cannabinoid compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Structurally Related Cannabinoid Compounds:

Cross-reactant	Concentration (ng/mL)	Cross-reactivity
Δ^9-THC	4	Positive

Structurally Unrelated Pharmacological Compounds:

Cross-reactant	Concentration (µg/mL)	Cross Reactivity
Acetaminophen	100	Negative
Acetylsalicyclic acid	60	Negative
Amorbarbital	100	Negative
l-Amphetamine	100	Negative
Benzoylecognine	100	Negative
Benzphetamine	200	Negative
Bromopheniramine	200	Negative
Bupropion	250	Negative
Buspirone	250	Negative
Chlorpheniramine	250	Negative
Caffeine	300	Negative
Codeine	250	Negative
Chlorpromazine	300	Negative
Dextromethorphen	300	Negative
Doxepine	200	Negative
Fenfluremine	1	Negative
3-OH Tyramine	250	Negative
Isoxsuprine	250	Negative
Meperidine	150	Negative
d-Ephedrine	200	Negative
l-Ephedrine	60	Negative
d-Pseudoephedrine	100	Negative
l-Pseudoephedrine	100	Negative
d-Phenylpropanolamine	250	Negative
l-Phenylpropanolamine	25	Negative
d,l-Phenylpropanolamine	25	Negative
Ranitidine	5	Negative
Valproic Acid	300	Negative
Tyramine	50	Negative
Trazodone	250	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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12. LZI Oral Fluid Sample Preparation Flow Chart. Lin-Zhi International, Inc. website. www.lin-zhi.com (2013).

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