LZI Cannabinoids (cTHC) 100 Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0070c (100/37.5 mL R_1/R_2 Kit)
0071c (1000/375 mL R_1/R_2 Kit)

2ºC 8ºC

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Cannabinoids (cTHC) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cannabinoids in human urine using 11-nor-Δ^9-THC-9-COOH, (the major metabolite of THC, referred to hereafter as cTHC). The calibrator at the cutoff has a value of 100 ng/mL. The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS or (2) permitting laboratories to establish quality control procedures. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. These assays are for prescription use only.

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

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 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, abstinence syndrome, and development of tolerance (4). At low dose, it produces mixed depressant and stimulant effects; at higher dose, marijuana acts as a CNS depressant (5-7).

In the past few decades, research has confirmed the presence of an endogenous endocannabinoid system or ECS (8). Endocannabinoids are produced within the human body and activate two known cannabinoid receptors, CB_1 and CB_2 (9). The CB_1 receptor is localized primarily to the brain and is thought to be responsible for the euphoric and anticonvulsive effects of cannabis, whereas the CB_2 receptor is found primarily in the immune system and thought to be responsible for the anti-inflammatory effects of Δ^9-THC (10-12).

Studies published in 2006 revealed that Δ^9-THC may actually induce cell death (13) and may even be a viable anti-tumor target (14). Due to the role the ECS may play in a number of physiological processes, much interest in the use of synthetic ECS ligands for therapeutic purposes remains high (15-17).

Δ^9-THC is easily absorbed by inhalation (smoking) or ingestion. 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 to a variety of compounds, the major one being the cTHC metabolite (6, 7). Approximately 70 % of THC is excreted in feces and urine within 72 hours of administration (18).

Assay Principle

The LZI Cannabinoids assay is a homogeneous enzyme immunoassay with 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 (19). 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, cannabinoid derivative-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; the unbound cannabinoid derivative-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-cannabinoid antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with cannabinoid in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and Controls are 5 mL, sold separately and contain negative human urine with sodium azide as a preservative.*

THC 100 Calibrators	REF
THC Negative Calibrator	0002c
THC Low Calibrator: Contains 50 ng/mL cTHC metabolite	0075c
THC Cutoff Calibrator: Contains 100 ng/mL cTHC metabolite	0077c
THC Intermediate Calibrator: Contains 150 ng/mL cTHC metabolite	0078c
THC High Calibrator: Contains 200 ng/mL cTHC metabolite	0079c

THC 100 Controls	REF
THC Level 1 Control: Contains 75 ng/mL cTHC metabolite	0076c
THC Level 2 Control: Contains 125 ng/mL cTHC metabolite	0009c

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 (20).*
- *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 (21-23). Use of plastics such as polyethylene is recommended (24). 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 (25, 26). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown Δ^9-THC analytes in urine are stable at -20ºC for up to 70 days (26-28). 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. If other instruments are used, performance will need to be validated by the laboratory (29, 30).

Assay Procedure

Analyzers with the 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 8 µL sample, 200 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) in 37ºC incubation temperature, 30-35 reading frames, and a 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 if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring of the cutoff level: use the 75 ng/mL and 125 ng/mL for the 100 ng/mL cutoff level.

Calibration and Quality Control

Good laboratory practices recommend the use of both a positive and 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 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 number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator which contains 100 ng/mL of cTHC 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 cTHC in the sample may then be estimated from the calibration curve.

Interpretation: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS or (2) permitting laboratories to establish quality control procedures.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of cannabinoids.*
2. *The test is not intended for quantifying these single analytes in samples.*
3. *A preliminary 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. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
7. *The test is designed for use with human urine only.*
8. *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: Typical results (ΔmA/min) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	409.7	2.3	0.6 %	409.7	3.6	0.9 %
25 ng/mL	435.1	2.6	0.6 %	435.1	4.2	1.0 %
50 ng/mL	464.6	3.6	0.8 %	464.6	5.0	1.1 %
75 ng/mL	498.6	3.4	0.7 %	498.6	5.1	1.0 %
100 ng/mL	535.7	3.1	0.6 %	535.7	4.6	0.9 %
125 ng/mL	570.9	3.4	0.6 %	570.9	5.3	0.9 %
150 ng/mL	601.9	4.8	0.8 %	601.9	6.2	1.0 %
175 ng/mL	623.8	3.1	0.5 %	623.8	4.8	0.8 %
200 ng/mL	635.8	2.8	0.4 %	635.8	4.6	0.7 %

100 ng/mL Cutoff Result:		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
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

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	2.4	2.9	116.6 %	2.3	3.3	147.3 %
25 ng/mL	28.4	2.4	8.4 %	28.3	2.8	9.8 %
50 ng/mL	50.0	2.0	3.9 %	49.9	2.2	4.4 %
75 ng/mL	71.5	2.2	3.1 %	71.5	2.7	3.8 %
100 ng/mL	97.0	2.1	2.2 %	97.0	2.5	2.6 %
125 ng/mL	125.1	3.2	2.5 %	125.2	3.4	2.7 %
150 ng/mL	155.2	3.2	2.1 %	155.1	3.7	2.4 %
175 ng/mL	178.8	4.0	2.2 %	178.8	4.6	2.6 %
200 ng/mL	196.7	4.7	2.4 %	196.6	5.3	2.7 %

100 ng/mL Cutoff Result:		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
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	22 Neg	88	78 Neg/ 10 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

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine with 95 % confidence, was tested to be 15 ng/mL for THC 100 in both qualitative and semi-quantitative analyses.

Accuracy: Forty-two (42) negative and 40 positive specimens for a total of 82 unaltered clinical urine specimens were tested with the LZI Cannabinoids (cTHC) Enzyme Immunoassay at the 100 ng/mL cutoff and confirmed with GC/MS or LC/MS. Specimens having a concentration of cTHC greater than 100 ng/mL by GC/MS or LC/MS were defined as positive, and specimens with lower concentrations by GC/MS or LC/MS were defined as negative in the tables below. The correlation results are summarized as follows (near cutoff samples are defined as ± 50 % of the cutoff value):

THC 100 - Semi-Quantitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	0	4*	15	25	100.0 %
Negative	2	30	6	0	0	90.5 %

THC 100 - Qualitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive *	0	0	4*	15	25	100.0 %
Negative *	2	30	6	0	0	90.5 %

Summary of Discordant Results in Semi-Quantitative Mode:

	Cutoff Value	LZI cTHC EIA (Neg/Pos)	cTHC GC/MS Value (ng/mL)
Semi-Quantitative Mode	100 ng/mL	Positive	83.0
		Positive	83.0
		Positive	85.0
		Positive	96.0

Summary of Discordant Results in Qualitative Mode :

	Cutoff Value	LZI cTHC EIA (Neg/Pos)	cTHC GC/MS Value (ng/mL)
Qualitative Mode	100 ng/mL	Positive	83.0
		Positive	83.0
		Positive	85.0
		Positive	96.0

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

$y = 0.9645x + 5.186, r^2 = 0.9976$

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100	0	1.9	N/A
92.5	15	21.5	143.2
80	40	44.8	112.0
70	60	62.4	104.0
60	80	80.0	10.0
50	100	103.2	103.2
40	120	128.1	106.7
30	140	137.5	98.2
20	160	157.3	98.3
10	180	181.3	100.7
0	200	195.2	97.6

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually 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 Cannabinoids (cTHC) Compounds:

Compound	Target [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-Reactivity
8-β-Hydroxy-Δ^9-THC	140	85.0	60.7 %
8-β-11-Dihydroxy- Δ^9-THC	160	71.7	44.8 %
Cannabidiol	16,000	84.3	0.5 %
Cannabinol	400	98.0	24.5 %
exo-THC	160	96.0	60.0 %
l-11-Hydroxy- Δ^9-THC	100	101.5	101.5 %
l-11-Nor-Δ^9-THC-9-Carboxylic Acid	100	97.4	97.4 %
l-11-Nor-Δ^9-THC-9-Carboxylic Acyl-Glucuronide	6000	90.2	1.5 %
Δ^8-THC	160	98.6	61.6 %
Δ^9-THC	260	94.9	36.5 %

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-Reactivity
Acetaminophen	500,000	6.6	0.001 %
Acetylsalicylic Acid	500,000	3.0	0.001 %
Amitryptyline	500,000	4.9	0.001 %
Amobarbital	500,000	2.6	0.001 %
Amphetamine	500,000	9.7	0.002 %
Benzoylecgonine	500,000	10.3	0.002 %
Bupropion	500,000	10.4	0.002 %
Caffeine	500,000	6.1	0.001 %
Chlorpheniramine	500,000	8.8	0.002 %
Chlorpromazine	500,000	3.8	0.001 %
Cocaine	500,000	8.6	0.002 %
Codeine	500,000	7.2	0.001 %
Dextromethorphan	500,000	10.2	0.002 %
Ecgonine Methyl Ester	500,000	6.9	0.001 %
d,l-Ephedrine	500,000	10.3	0.002 %
Imipramine	500,000	9.1	0.002 %
JWH-018(1-pentyl-3 (1-naphthoyl)indole)	500,000	16.0	0.003 %
JWH-073(1-butyl-3 (1-naphthoyl)indole)	500,000	24.0	0.005 %
Lidocaine	500,000	10.1	0.002 %
Meperidine	500,000	12.8	0.003 %
Methadone	500,000	10.4	0.002 %
Methamphetamine	500,000	7.4	0.001 %
Methaqualone	500,000	13.8	0.003 %
Morphine	500,000	9.0	0.002 %
Nortriptyline	500,000	8.5	0.002 %
Oxazepam	500,000	9.6	0.002 %
Phencyclidine	500,000	9.6	0.002 %
Phenobarbital	500,000	10.9	0.002 %
Promethazine	500,000	10.1	0.002 %

Structurally Unrelated Pharmacological Compounds, continued:

Compound	Target [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-reactivity
Propoxyphene	500,000	9.3	0.002 %
Ranitidine	500,000	9.7	0.002 %
Secobarbital	500,000	5.7	0.001 %
Valproic Acid	500,000	9.9	0.002 %

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

Interference: Endogenous Substances

The following endogenous compounds were spiked into a pool of processed negative urine (cannabinoids free urine) to the desired concentrations listed in the table below. Standards of cTHC were then spiked into the pools of processed urine containing the endogenous compounds to the concentrations listed below as positive or negative controls. Results indicate that there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff values of 100 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (mg/dL)	0 ng/mL (ng/mL)	75 ng/mL Control (ng/mL)	125 ng/mL Control (ng/mL)
None	N/A	0.0	66.8	115.4
Acetone	1000	0.0	65.6	120.7
Ascorbic Acid	500	0.0	62.4	110.1
Creatinine	500	0.0	64.6	123.4
Ethanol	1000	0.0	65.5	127.5
Galactose	10	0.0	64.8	118.8
γ-Globulin	500	0.0	68.1	115.7
Glucose	1500	0.0	67.6	117.1
Hemoglobin	300	0.0	67.3	117.4
Human Serum Albumin	500	0.0	66.8	123.7
Oxalic Acid	100	0.0	60.4	105.6
Riboflavin	0.65	2.7	75.5	124.3
Sodium Chloride	2000	0.0	66.8	118.0
Urea	2000	0.0	66.1	116.1
pH 3	N/A	0.0	58.5	108.7
pH 4	N/A	0.0	67.3	124.4
pH 5	N/A	3.2	70.8	131.5
pH 6	N/A	2.3	72.0	135.2
pH 7	N/A	4.3	74.8	143.6
pH 8	N/A	0.0	76.8	144.1
pH 9	N/A	2.5	79.6	136.1
pH 10	N/A	4.7	76.5	136.8
pH 11	N/A	0.0	71.5	121.0

Specific Gravity: Samples ranging in specific gravity from 1.002 to 1.025 were tested with the assay in the presence of 0 ng/mL, 75 ng/mL, and 125 ng/mL (positive and negative controls for THC 100) of cTHC, and no interference was observed.

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