LZI Cotinine II Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0520 (100/37.5 mL R_1/R_2 Kit)
0521 (1000/375 mL R_1/R_2 Kit) 2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The LZI Cotinine II Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cotinine in human urine at the cutoff value of 200 ng/mL when calibrated against cotinine. The assay is intended as an aid in the detection of cotinine after use or exposure to tobacco products. 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 confirmation 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 method (e.g., gas or liquid chromatography and mass spectrometry) must be used in order 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

Nicotine is the primarily addictive compound in tobacco products (3). For the past three decades, there has been a tremendous attention to tobacco smoking and the so-called "passive inhalation" of tobacco smoke due to its correlation to lung cancer.

Inhaled tobacco smoke reaches small airways and alveoli of the lungs, where 90 % of nicotine is absorbed. When nicotine is absorbed, it is readily metabolized into cotinine by the liver (4). Urine concentrations of both nicotine and cotinine correlate with cigarette use in active smokers (5). While nicotine has a very short half-life of approximately 40 minutes (6), cotinine has an average half-life of 20 hours (7), and can be detected in the urine of a smoker even several days after the smoking has ceased.

Several methods have been used to determine the smoking status of an individual. These include measurement of thiocyanate, carbon monoxide, and cotinine. Measurement of both thiocyanate and carbon monoxide, however, is more likely to be affected by environmental factors and can cause false positive results. Since cotinine can only be derived from metabolism of nicotine, it is a better marker for determination of the smoker status.

A number of studies have shown second hand smoke concentrations for non-smokers fall below a 200 ng/mL cotinine urinary concentration (8), but some individuals that are highly exposed to environmental tobacco smoke can have urinary cotinine concentrations exceeding 200 ng/mL (9).

Assay Principle

The LZI Cotinine II 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 (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, cotinine-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 would bind to free drug; the unbound cotinine-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 primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a mouse monoclonal anti-cotinine 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 cotinine 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.*

COTININE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 100 ng/mL cotinine	0522
Cutoff Calibrator: Contains 200 ng/mL cotinine	0523
Intermediate Calibrator: Contains 400 ng/mL cotinine	0524
High Calibrator: Contains 1000 ng/mL cotinine	0525

COTININE Controls	REF
Level 1 Control: Contains 150 ng/mL cotinine	0527
Level 2 Control: Contains 250 ng/mL cotinine	0528

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 (11).*
- *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

Urine samples may be collected in plastic or glass containers. Some plastics may adsorb drugs. Use of plastics such as polyethylene is recommended (12). 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 (13, 14). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown cotinine analytes in urine are stable at -20ºC for up to seven weeks (14, 15). 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 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 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 Beckman Coulter AU480. If other instruments are used, performance will need to be validated by the laboratory (16, 17).

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 points, FIXED method, and 340 nm primary wavelength.

For qualitative analysis use the 200 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: 150 ng/mL and 250 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 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 200 ng/mL of cotinine, 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 a preliminary 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 cotinine in the sample may then be estimated from the calibration curve.

Limitations

1. *Boric Acid at 1% w/v and Citric Acid may cause false negative results. Boric Acid and Citric Acid are not recommended as a preservative for urine.*
2. *A preliminary positive result from this assay indicates only the presence of cotinine and does not necessarily correlate with the extent of physiological and psychological effects (e.g., intoxication). This test is not intended for quantifying the individual analyte 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 abuse 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 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 Beckman Coulter AU480 automated chemistry analyzer.

Precision:

Semi-Quantitative Analysis: The following concentrations were determined with reference curves from five calibrators. Typical results were measured in ng/mL. Positive/Negative results are as follows:

200 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Cotinine Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
50 ng/mL	25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	50.0 %	22	22 Neg	88	88 Neg
150 ng/mL	75.0 %	22	22 Neg	88	88 Neg
200 ng/mL	100.0 %	22	14 Neg/ 8 Pos/	88	55 Neg/ 33 Pos/
250 ng/mL	125.0 %	22	22 Pos	88	88 Pos
300 ng/mL	150.0 %	22	22 Pos	88	88 Pos
350 ng/mL	175.0 %	22	22 Pos	88	88 Pos
400 ng/mL	200.0 %	22	22 Pos	88	88 Pos

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

200 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Cotinine Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
50 ng/mL	25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	50.0 %	22	22 Neg	88	88 Neg
150 ng/mL	75.0 %	22	22 Neg	88	88 Neg
200 ng/mL	100.0 %	22	10 Neg/ 12 Pos	88	51 Neg/ 37 Pos
250 ng/mL	125.0 %	22	22 Pos	88	88 Pos
300 ng/mL	150.0 %	22	22 Pos	88	88 Pos
350 ng/mL	175.0 %	22	22 Pos	88	88 Pos
400 ng/mL	200.0 %	22	22 Pos	88	88 Pos

Accuracy: One-hundred and four (104) unaltered clinical urine specimens were tested with the LZI Cotinine II Enzyme Immunoassay and confirmed by GC/MS. Specimens having a cotinine concentration greater than or equal to 200 ng/mL by GC/MS are defined as positive and specimens with concentrations below 200 ng/mL by GC/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:

200 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	0	1*	11	16	96.4 %
Negative	20	32	23	1**	0	98.7 %

The following table summarizes the result for the discordant samples:

200 ng/mL Cutoff	COT GC/MS (ng/mL)	GC/MS Result	LZI COT II EIA Result
Sample 57*	128.6	-	+
Sample 78**	204.2	+	-

Qualitative Accuracy Study:

200 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	0	1*	11	16	96.4 %
Negative	20	32	23	1**	0	98.7 %

The following table summarizes the result for the discordant samples:

200 ng/mL Cutoff	COT GC/MS (ng/mL)	GC/MS Result	LZI COT II EIA (mAU)	LZI EIA	Qualitative Cutoff Rate (mAU)
Sample 57*	128.6	-	146.6	+	126.4
Sample 78**	204.2	+	93.8	-	126.4

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section) of the entire assay range, a drug-free urine pool spiked with cotinine at 1000 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. Samples from the linear range of the assay (100 ng/mL to 1000 ng/mL) were tested with recovery ranging from 97.9 % to 110.8 %.

Target Concentration (ng/mL)	Determined (ng/mL)	% Recovery
1000	978.9	97.9 %
900	919.6	102.2 %
800	851.7	106.5 %
700	766.0	109.4 %
600	664.8	110.8 %
500	539.1	107.8 %
400	395.0	98.8 %
300	302.4	100.8 %
200	199.9	100.0 %
100	109.0	109.0 %
0	19.3	N/A

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 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 with results below the cutoff value were listed as Not Detected (ND).

Cotinine:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
(-)-Cotinine	200	100.00 %

Cotinine Metabolites:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
(+)-Anabasine	250000	0.08 %
S(-)-Nicotine	25000	0.80 %
Nicotinic Acid	100000	0.20 %
(-) Norcotinine	1000	20.00 %
(+) Norcotinine	100000	0.20 %
(R, S)-Norcotinine	850	23.53 %
(+/-)-Nornicotine	250000	0.08 %
trans-3'-hydroxycotinine	10000	2.00 %

Structurally Unrelated Compounds:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Acetaminophen	100000	ND	Neg	Pos
6-Acetylmorphine	10000	ND	Neg	Pos
Acetylsalicylic Acid	100000	ND	Neg	Pos
Amitriptyline	100000	ND	Neg	Pos
Amlodipine Besylate	100000	ND	Neg	Pos
Amoxicillin	100000	ND	Neg	Pos
Azelastine	100000	ND	Neg	Pos
d-Amphetamine	100000	ND	Neg	Pos
Atorvastatin	20000	ND	Neg	Pos
Benzoylecgonine	100000	ND	Neg	Pos
Brompheniramine	100000	ND	Neg	Pos
Buprenorphine	15000	ND	Neg	Pos
Bupropion	100000	ND	Neg	Pos
Caffeine	100000	ND	Neg	Pos
Carbamazepine	100000	ND	Neg	Pos
Carbinoxamine	100000	ND	Neg	Pos
Cetirizine	100000	ND	Neg	Pos
Chlorpheniramine	100000	ND	Neg	Pos
Chlorpromazine	100000	ND	Neg	Pos
Clemastine	100000	ND	Neg	Pos
Clomipramine	100000	ND	Neg	Pos
Codeine	100000	ND	Neg	Pos
Cyproheptadine	100000	ND	Neg	Pos
Desipramine	100000	ND	Neg	Pos
Desloratadine	100000	ND	Neg	Pos
Dexchlorpheniramine	100000	ND	Neg	Pos
Diphenhydramine	100000	ND	Neg	Pos
Doxylamine	100000	ND	Neg	Pos
Duloxetine	100000	ND	Neg	Pos
Emedastine	100000	ND	Neg	Pos
Fentanyl (citrate)	10000	ND	Neg	Pos
Fexofenadine	100000	ND	Neg	Pos
Fluoxetine	100000	ND	Neg	Pos
Fluphenazine	100000	ND	Neg	Pos
Gabapentin	100000	ND	Neg	Pos
Hydrocodone	100000	ND	Neg	Pos
Hydromorphone	100000	ND	Neg	Pos
Hydroxyzine	100000	ND	Neg	Pos
Ibuprofen	100000	ND	Neg	Pos
Imipramine	100000	ND	Neg	Pos
Lisinopril	100000	ND	Neg	Pos
Levocabastine	100000	ND	Neg	Pos
Losartan	10000	ND	Neg	Pos
Loratadine	100000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100000	ND	Neg	Pos
MDEA	100000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100000	ND	Neg	Pos
Meperidine	100000	ND	Neg	Pos
Metformin	100000	ND	Neg	Pos

Structurally Unrelated Compounds, continued:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Methapyrilene	100000	ND	Neg	Pos
Metoprolol	100000	ND	Neg	Pos
Methadone	100000	ND	Neg	Pos
d-Methamphetamine	100000	ND	Neg	Pos
Morphine	100000	ND	Neg	Pos
Nortriptyline	100000	ND	Neg	Pos
Omeprazole	100000	ND	Neg	Pos
Oxazepam	100000	ND	Neg	Pos
Oxycodone	100000	ND	Neg	Pos
Oxymorphone	100000	ND	Neg	Pos
Phenobarbital	100000	ND	Neg	Pos
Promethazine	100000	ND	Neg	Pos
(1S,2S)-(+)Pseudoephedrine	100000	ND	Neg	Pos
Quetiapine	100000	ND	Neg	Pos
Ranitidine	100000	ND	Neg	Pos
Salbutamol (Albuterol)	100000	ND	Neg	Pos
Sertraline	100000	ND	Neg	Pos
Terfenadine	100000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1000	ND	Neg	Pos
l-Thyroxine	10000	ND	Neg	Pos
Tramadol	100000	ND	Neg	Pos
Triprolidine	100000	ND	Neg	Pos
Zolpidem	10000	ND	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 Preservative Compound Interference Study:
The following endogenous compounds were spiked into negative urine and the two levels of controls (150 ng/mL and 250 ng/mL) for the assay. These spiked solutions were then evaluated in semi-quantitative and qualitative modes.
Interference was observed with Boric Acid and Citric Acid. No other major interference was found as all other spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 200 ng/mL. Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked Cotinine Concentration		
		0 ng/mL	150 ng/mL Control	250 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 ($CaCl_2$)	300	Neg	Neg	Pos
Citric Acid (pH 3)*	800	Neg	Neg	Neg
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

The following endogenous compounds which showed interference at ±25 % of the cutoff concentrations were then spiked into negative urine at ±50 % of the cutoff concentrations (100 ng/mL and 300 ng/mL) for the assay. Interference was still observed with Boric Acid and Citric Acid. Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked Cotinine Concentration		
		0 ng/mL	100 ng/mL	300 ng/mL
Boric Acid	1000	Neg	Neg	Neg
Citric Acid (pH 3)	800	Neg	Neg	Neg

pH Interference Study: Negative urine and urine spiked with analyte to the two levels of controls (150 ng/mL and 250 ng/mL) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were then evaluated in semi-quantitative and qualitative modes.
No major interference with these pH levels was observed as all pH adjusted levels gave correct corresponding preliminary positive/negative results against the cutoff value of 200 ng/mL. Results are summarized in the following table:

pH	Spiked Cotinine Concentration		
	0 ng/mL	150 ng/mL Control	250 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 1.005 to 1.028 were split into three portions each and either left un-spiked or further spiked to a final cotinine concentration of either 150 ng/mL or 250 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No interference was observed.

Open-Vial Reagent and Calibrator/Control Stability: Real-time data for open-vial reagent and calibrator/control stability studies at Cold Temperature (2-8ºC) have been carried out up to Day 730. Results from open-vial studies indicate that degradation is minimal up to Day 730, and, based on the real-time data, suggests an open-vial stability of up to 24 months. Open-vial reagents and calibrators/controls should be stored at 2-8 ºC for maximum shelf life.

Closed-Vial Calibrator/Control Stability: Real-time data for closed-vial calibrator/control stability studies at Cold Temperature (2-8ºC) have been carried out up to Day 730 and remain on-going. Results from closed-vial studies indicate that degradation is minimal at Cold Temperature (2-8ºC) up to Day 730 in comparison to Day 1. Closed-vial calibrators/controls should be stored at 2-8ºC for maximum shelf life.

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
Rx ONLY	Medical Prescription Only		

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A point (period/stop) is always used in this instruction for use document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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For technical assistance please call: (408) 970-8811

Manufacturer:
Lin-Zhi International, Inc.
2945 Oakmead Village Court
Santa Clara, CA 95051 USA
Tel: (408) 970-8811
Fax: (408) 970-9030
www.lin-zhi.com

Authorized European Rep. within the EU:
CEpartner4U
Esdoornlaan 13
3951 DB Maarn
The Netherlands
www.cepartner4u.eu

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