LZI Ethyl Glucuronide Enzyme Immunoassay – EU Only

IVD For In Vitro Diagnostic Use Only

REF 0490b (100/37.5 mL R_1/R_2 Kit)
0491b (1000/375 mL R_1/R_2 Kit) 2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Ethyl Glucuronide (EtG) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of ethyl glucuronide in human urine at a cutoff value of 1000 ng/mL. The assay is designed for 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

Ethyl Glucuronide (EtG) is an ethanol metabolite that is formed from the conjugation of ethanol to glucuronic acid (3, 4). Although EtG is a minor metabolite of ethanol, composing less than 0.05 % of the ingested ethanol dose (4, 5), its long detection time has made it an increasingly popular biomarker for alcohol consumption (6-9). Due to rapid metabolism and excretion, the time frame for alcohol detection in urine is normally less than 12 hours (10). Depending on the dosage of ethanol ingested, EtG has a detection window of up to four days following elimination of ethanol from the body (4, 6, 7, 11-13).

EtG can be used to screen for recent alcohol intake as well as chronic alcoholism due to its non-volatile and water-soluble properties (14-18).

Assay Principle

The LZI Ethyl glucuronide Enzyme Imunnoaassay 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 (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, ethyl glucuronide-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 ethyl glucuronide-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 rabbit polyclonal anti-EtG 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 EtG 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.*

ETHYL GLUCURONIDE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: 250 ng/mL ethyl glucuronide	0492b
Cutoff Calibrator: 1000 ng/mL ethyl glucuronide	0493b
Intermediate Calibrator #1: 2000 ng/mL ethyl glucuronide	0494b
Intermediate Calibrator #2: 3000 ng/mL ethyl glucuronide	0495b
High Calibrator: 4000 ng/mL ethyl glucuronide	0496b

ETHYL GLUCURONIDE Controls	REF
Level 1 Control: 500 ng/mL ethyl glucuronide	0497b
Level 2 Control: 1500 ng/mL ethyl glucuronide	0498b

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.*

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 (21). Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (22). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown ethyl glucuronide analytes in urine are stable frozen at -20ºC up to six months (23, 24). Samples should be at a 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 (25, 26).

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 20 µL sample, 180 µL of antibody reagent (R_1), and 60 µL of enzyme conjugate reagent (R_2) at 37°C incubation temperature, 26-30 reading points, and a 340 nm primary wavelength. For qualitative analysis, use the 1000 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all six 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: 500 ng/mL and 1500 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 guidelines and regulations.

Results

Note: A positive test result does not always mean a person ingested alcohol and a negative test result does not always mean a person did not ingest alcohol. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator which contains 1000 ng/mL of EtG 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 calibrator used as the cutoff is considered a preliminary positive. A sample with a change in absorbance (ΔmA/min) lower than that obtained with the calibrator used as the cutoff 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 six calibrators. The concentration of ethyl glucuronide 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 ethyl glucuronide. The test is not intended for quantifying this single analyte in samples.*
2. *A preliminary positive result does not necessarily indicate alcohol ingestion.*
3. *A negative result does not necessarily mean a person did not ingest alcohol.*
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 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.

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 summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 1000 ng/mL ethyl glucuronide cutoff (as positive) or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator (as negative).

Structurally Related Ethyl glucuronide Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Acetylaldehyde	>1000	Negative
Buprenorphine Glucuronide	>50	Negative
Butanol	>1000	Negative
D-Glucose	>1000	Negative
Ethanol	>1000	Negative
Ethylene Glycol	>1000	Negative
Glucuronic Acid	>1000	Negative
Isopropanol	>1000	Negative
Lorazepam-glucuronode	20	Negative
Methanol	>1000	Negative
Methyl-glucuronide	25	Negative
Morphine-3-glucuronide	>50	Negative
Morphine-6-glucuronide	>100	Negative
N-Propanol	>1000	Negative
Nitrophenyl-glucuronide	>1000	Negative
Norbuprenorphine Glucuronide	>50	Negative
Oxazepam-glucuronide	>50	Negative
Temazepam-glucuronide	>50	Negative
Phenyl-b-D-glucuronide	>1000	Negative

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
6-AM	>250	Negative
Acetaminophen	>500	Negative
Acetylsalicyclic Acid	>500	Negative
Amitryptyline	>100	Negative
Amoxicillin	>100	Negative
Amphetamine	>1000	Negative
Benzoylecgonine	>1000	Negative
Caffeine	>100	Negative
Carbamazepine	>500	Negative
Chlorpromazine	>100	Negative
Clomipramine	>100	Negative

Structurally Unrelated Pharmacological Compounds, continued:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Cimetidine	>500	Negative
Codeine	>1000	Negative
Desipramine	>1000	Negative
Dextromethorphan	>250	Negative
Dihydrocodeine	>500	Negative
Doxepine	>200	Negative
Ephedrine	>1000	Negative
Fentanyl	>200	Negative
Fluoxetine	>1000	Negative
Fluphenazine	>500	Negative
Hydrocodone	>200	Negative
Hydromprphone	>200	Negative
Ibuprofen	>1000	Negative
Imipramine	>1000	Negative
Levorphanol	>500	Negative
Lorazepam	>1000	Negative
Maprotiline	>1000	Negative
Meperidine	>1000	Negative
Methadone	>1000	Negative
Metronidazole	>500	Negative
Morphine	>1000	Negative
Nalbuphine	>1000	Negative
Naltrexone	2500	Negative
Norcodeine	>500	Negative
Normorphine	>500	Negative
Nortryptyline	>1000	Negative
Oxazepam	>500	Negative
Oxycodone	>500	Negative
Phenobarbital	>1000	Negative
Ranitidine	>500	Negative
Secobarbital	>1000	Negative
Talwin	>500	Negative
Thioridazine	>500	Negative
Tramadol	>500	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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