LZI Oral Fluid Ethyl Alcohol Enzymatic Assay

REF S0220 (75/37.5 mL R_1/R_2 Kit)
S0221 (750/375 mL R_1/R_2 Kit) 2ºC 8ºC

For Forensic Use Only

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Ethyl Alcohol Enzymatic Assay is a homogeneous enzymatic assay intended for the quantitative determination of ethyl alcohol in neat human oral fluid, collected into an LZI Oral Fluid Collector. The assay is designed for use with a number of automated clinical chemistry analyzers. This is a non-FDA approved assay for Forensic Use Only and as such should not be repackaged for *in vitro* diagnostic use.

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 alcohol can be found in regular alcoholic liquors, a variety of foods, drinks, candies, and medicinal preparations. When alcohol is ingested, it quickly spreads to the whole body and the majority (>90 %) is metabolized in the liver and excreted. After ingestion, alcohol can be found in human oral fluid, urine, and serum (3).

Alcohol intoxication can lead to severe loss of alertness, stupor, coma, and death, and frequently causes public safety issues. It can also lead to birth defects such as fetal alcohol syndrome (3-7).

Alcohol concentration in urine is commonly used for measuring legal impairment, forensic judgment, diagnosis, treatment of alcohol dependency, and detection of alcohol intoxication. Many different methods are available for determining alcohol concentration (3, 4) in biological fluid.

Assay Principle

The LZI Oral Fluid Ethyl Alcohol Enzymatic Assay is a set of homogeneous ready-to-use liquid reagents based on the unique enzymatic reaction of alcohol dehydrogenase (ADH). In the presence of nicotinamide adenine dinucleotide (NAD), ADH converts ethyl alcohol to acetaldehyde and reduces NAD to NADH. On the other hand, when ethyl alcohol is absent, ADH has limited enzymatic activity with very little absorbance change at 340 nm. The ethyl alcohol concentration is directly proportional to the ADH activity. The enzyme activity is measured at 340 nm wavelength.

$$\text{Ethyl Alcohol} + \text{NAD} \xleftrightarrow{\text{ADH}} \text{NADH} + \text{Acetaldehyde}$$

Reagents Provided

Buffer Reagent (R_1): Contains tris-based buffer with sodium azide (0.09 %) as a preservative.

Enzyme Reagent (R_2): Contains alcohol dehydrogenase (ADH), nicotinamide adenine dinucleotide (NAD), stabilizers, and 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 ETHYL ALCOHOL (OF EtOH) Calibrators/Controls	REF #
Oral Fluid Negative Calibrator	S0001
25 mg/dL Control/Calibrator: Contains 25 mg/dL (0.025 %) ethyl alcohol	S0226
50 mg/dL Calibrator: Contains 50 mg/dL (0.05 %) ethyl alcohol	S0223
75 mg/dL Control/Calibrator: Contains 75 mg/dL (0.075 %) ethyl alcohol	S0227
100 mg/dL Calibrator: Contains 100 mg/dL (0.10 %) ethyl alcohol	S0228

Collector**

*** Collector is 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 Forensic 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 (7).*
- *Do not use the reagents beyond their expiration dates.*
- *Calibrators and controls should be stored tightly capped inside a refrigerator.*

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 ethyl alcohol is stable in oral fluid. No further study was conducted beyond 21 days.

Samples to be shipped 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) (8).

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.

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 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. 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.

Calibration and Quality Control

Good laboratory practices recommend the use of control specimens to ensure proper assay performance. The negative, 50 mg/dL, and 100 mg/dL calibrators should be used for calibration of the assay for each batch of samples. Use controls to validate the calibration. Recalibrate the instrument whenever different bottles of reagents are used or if there is a change in calibrators or reagent lot.

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.

Limitations

1. *The legal alcohol intoxication level varies by region and age group.*
2. *The test result should be interpreted in light of clinical signs and symptoms.*
3. *Ethyl alcohol is volatile. Precaution is suggested during specimen collection and required to prevent alcohol evaporation from calibrators, controls, and samples.*
4. *The test is designed for use with human oral fluid only.*

Typical Performance Characteristics

Specificity: Various compounds that are structurally related to ethyl alcohol were tested for cross reactivity. Levels tested exceed toxic concentrations. Therefore, interference is not clinically significant.

Structurally Related Ethyl Alcohol Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Acetaldehyde	2000	0.0 %
Acetone	2000	0.0 %
n-Butanol	2000	1.5 %
Ethylene Glycol	2000	0.0 %
Isopropanol	2000	0.0 %
Methanol	2000	0.0 %
n-Propanol	2000	11.0 %

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

Endogenous Substance: Grossly hemolyzed (800 mg/dL hemoglobin), icteric (30 mg/dL bilirubin), and lipemic (1000 mg/dL triglycerides) samples were found to have no interference with the assay.

Bibliography:

1. Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, (1986).
2. Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **53**(69): 11970 (1988).
3. Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man.* 3rd edition, Chicago, IL. Year Book Medical Publishers Inc., 322-324 (1989).
4. Beutler, H.O., Ethanol. In: Bergmeyer HU, ed. *Methods of Enzyme Analysis*, Vol VI. 3rd ed, New York: Academic Press, 598-606 (1984).
5. Wyngaarden, J.B., Smith, L.H. Jr, eds. *Cecil Textbook of Medicine*. Philadelphia, PA: WB Saunders Co; 48-52 (1988).
6. Ellenhorn, M.J., Barceloux, D.G., *Medical Toxicology*. New York, NY: Elsevier Science Publishing Company, Inc., 525-526 & 782-796 (1988).
7. Tietz, N.W., ed. Textbook of Clinical Chemistry. Philadelphia, PA: WB Saunders Co; 1704-1706 & 1692-1694 (1986).
8. Sodium Azide. National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html
9. LZI Oral Fluid Sample Preparation Sheet.

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