

LZI Oral Fluid Oxycodone Enzyme Immunoassay

REF S0240b (75/37.5 mL R₁/R₂ Kit)
S0241b (750/375 mL R₁/R₂ Kit)



FOR RESEARCH & DEVELOPMENT USE ONLY

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Oxycodone Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of oxycodone and its metabolite, oxymorphone, in neat human oral fluid, collected into an LZI Oral Fluid Collector, at the cutoff value of 40 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 oxycodone and oxymorphone 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

Oxycodone is a semi-synthetic narcotic analgesic prescribed for pain management in patients with moderate to severe pain. The drug is approximately equipotent with morphine, but has a higher oral/parenteral dose (3). Similar to morphine, oxycodone can produce drug tolerance and therefore has the potential of being abused. Oxycodone is metabolized by N- and O-demethylation. The oxymorphone metabolite is a potent narcotic analgesic and the noroxycodone is relatively inactive. 33-61 % of a single dose of oxycodone is excreted in the 24 hour urine as free (13-19 %) and conjugated oxycodone (7-29 %), conjugated oxymorphone (13-14 %) and an unknown amount of noroxycodone. Oral fluid normally contains parent drug rather than drug metabolites, which are most commonly detected in urine (4, 5).

Assay Principle

The LZI Oral Fluid Oxycodone 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 (6). 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, oxycodone-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 and the unbound oxycodone-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 (R₁): Contains mouse monoclonal anti-oxycodone antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R₂): Contains oxycodone-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 negative synthetic oral fluid matrix with sodium azide as a preservative.

ORAL FLUID OXYCODONE Calibrator/Control	REF #
Oral Fluid Negative Calibrator	S0001
Low Calibrator: Contains 20 ng/mL oxycodone	S0242b
Cutoff Calibrator: Contains 40 ng/mL oxycodone	S0243b
Intermediate Calibrator: Contains 60 ng/mL oxycodone	S0244b
High Calibrator: Contains 80 ng/mL oxycodone	S0245b
Level 1 Control: Contains 30 ng/mL oxycodone	S0247b
Level 2 Control: Contains 50 ng/mL oxycodone	S0248b

Collectors **

**Collectors are 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 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 stored refrigerated at 2-8°C 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 (2-8°C) for up to 21 days or frozen (-20°C) for up to 21 days. Studies have been performed up to 21 days to show oxycodone is stable in oral fluid. No further study was conducted beyond 21 days.

Samples should always be shipped cold (2-8°C), 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) (7).

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 (18-25°C) for testing.

Samples do not require dilution or any additional correction factors.

Fresh and properly stored oral fluid samples should be within the normal pH range of 6-8; however, any sample with pH ranging from 3-10 can be tested without any pretreatment of the samples.

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 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 45 µL sample, 150 µL of antibody reagent (R₁), and 75 µL of enzyme conjugate reagent (R₂) in 37°C incubation temperature, 30-35 reading frames, and 340 nm primary wavelength.

For qualitative analysis, use the 40 ng/mL cutoff calibrator. For semi-quantitative analysis, use all five calibrators and two controls. 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: 30 ng/mL and 50 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 number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 40 ng/mL of oxycodone, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance ($\Delta A/\text{min}$) equal to or greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance ($\Delta A/\text{min}$) 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 two controls.

Limitations

1. A positive result from the assay indicates only the presence of oxycodone. The test is not intended for quantifying this single analyte 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 oxycodone.
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, endogenous or exogenous 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 a 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.

Structurally Related Oxycodone Compounds:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Oxycodone	40	96.90 %
Hydrocodone	4,500	1.00 %
Hydromorphone	3,000	1.40 %
Oxymorphone	35	114.00 %
Noroxycodone	350	12.09 %
Noroxymorphone	425	10.02 %
Codeine	200,000	0.00 %
Dextromethorphan	500,000	0.00 %
Dihydrocodeine	90,000	0.05 %
Levorphanol	12,000	0.35 %
Naloxone	10,000	0.27 %
Norcodeine	400,000	0.01 %
Morphine	100,000	0.00 %
Oxymorphone-glucuronide	23	173.90 %
Codeine-6-b-glucuronide	50,000	0.00 %
Morphine-3-glucuronide	60,000	0.03 %
6-AM	500,000	0.00 %
Norbuprenorphine	500,000	0.00 %

There is a possibility that metabolites of the compounds listed above may interfere with the oxycodone immunoassay and cause false results.

Structurally Unrelated Pharmacological Compounds:

Cross-reactant	Concentration (ng/mL)	% Cross Reactivity
Acetaminophen	500,000	0.00 %
Acetylsalicylic acid	500,000	0.00 %
Amobarbital	500,000	0.00 %
Benzoylcegonine	500,000	0.00 %
Brompheniramine	500,000	0.00 %
Bupropion	500,000	0.00 %
Caffeine	500,000	0.00 %
Chlorpheniramine	500,000	0.00 %
Chlorpromazine	500,000	0.00 %
d,l-Phenylpropanolamine (Phenethylamine)	500,000	0.00 %
d-Ephedrine	500,000	0.00 %
l-Ephedrine	500,000	0.00 %
d-Methamphetamine	500,000	0.00 %
Ecgonine (Ecgonine Methyl-ester)	500,000	0.00 %
Meperidine	500,000	0.00 %
Methadone	500,000	0.00 %
Nicotine	500,000	0.00 %
Norpropoxyphene	500,000	0.00 %
Phencyclidine	500,000	0.00 %
Promethazine	500,000	0.00 %
Propranolol	500,000	0.00 %
Secobarbital	500,000	0.00 %
Trazodone	500,000	0.00 %
Tyramine	500,000	0.00 %
Valproic acid	500,000	0.00 %

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

Bibliography:

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7. LZI Oral Fluid Sample Preparation Sheet.

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