LZI Fentanyl Enzyme Immunoassay – EU Only

IVD For In Vitro Diagnostic Use Only

REF 0590 (100/37.5 mL R_1/R_2 Kit)
0591 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The LZI Fentanyl II Enzyme Immunoassay is an in vitro diagnostic test intended for the qualitative and semi-quantitative determination of norfentanyl in human urine at the cutoff value of 5 ng/mL when calibrated against norfentanyl. 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 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

Fentanyl is an important opioid analgesic used widely in surgical operations and is a controlled substance (3). Fentanyl is most commonly encountered in the form of patches applied to the skin, as "lollipops" which can be dissolved in the mouth through the mucous membrane, or can be administered intravenously. It is 50-100 times stronger than morphine (4, 5) and cases of fentanyl abuse via intravenous injection, inhalation, oral, or nasal applications have been previously reported (6). Fentanyl is used in the treatment of acute and chronic pain, usually in patients who no longer respond to high doses of less potent opioids such as morphine or oxycodone. Due to its potency and wide availability as a prescribed drug, fentanyl has been abused and misused by health professionals, pain management patients, and recreational abusers (7).

Due to its short elimination half-life and approximately 90 % metabolism, fentanyl is difficult to detect in urine (8). Fentanyl undergoes extensive hepatic biotransformation to metabolites coming from hydrolysis, N-dealkylation, or hydroxylation reactions (9). In an intravenous dose of fentanyl, up to 85 % is excreted in urine over a three- to four- day period with 0.4-6 % eliminated as unchanged fentanyl and 26-55 % eliminated as the norfentanyl metabolite (10).

Fentanyl analogs also have high potency analgesic activities. Numerous reports have been published with modified fentanyl-related compounds abused as designer drugs (11-13).

Other recently available fentanyl analogs associated with abuse and severe intoxication include butyryl fentanyl and 4-fluorobutyryl fentanyl (14-18).

Assay Principle

The LZI Fentanyl assay 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, fentanyl-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 fentanyl-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 (R_1): Contains a mouse monoclonal anti-fentanyl 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 fentanyl 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.*

NORFENTANYL Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 2.5 ng/mL norfentanyl	0562
Cutoff Calibrator: Contains 5 ng/mL norfentanyl	0563
Intermediate Calibrator: Contains 10 ng/mL norfentanyl	0564
High Calibrator: Contains 20 ng/mL norfentanyl	0565

NORFENTANYL Controls	REF
Level 1 Control: Contains 3.75 ng/mL norfentanyl	0567
Level 2 Control: Contains 6.25 ng/mL norfentanyl	0568

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

Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to four weeks (21) or at room temperature for up to four weeks (21, 22). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown norfentanyl samples in urine are stable at -20ºC for up to six months (23). Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Samples should be equilibrated to 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 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.

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 20 µL sample, 120 µL of antibody reagent (R_1), 45 µL of enzyme conjugate reagent (R_2), 12-16 reading frame, FIXED method, and 340 nm primary wavelength. If additional washing steps are required, reference analyzer specific parameter sheet.

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: 3.75 ng/mL and 6.25 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 5 ng/mL of norfentanyl, is used as a reference for distinguishing a preliminary 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 norfentanyl in the sample may then be estimated from the calibration curve.

Limitations

1. *Boric Acid at 1% w/v may cause false negative results. Boric Acid is not recommended as a preservative for urine.*
2. *Dextromethorphan may cause false positive results at concentrations greater than 40,000 ng/mL.*
3. *A preliminary positive result from this assay indicates only the presence of norfentanyl 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 analytes in samples.*
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:

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

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.0	0.0	N/A	0.0	0.2	N/A
1.25 ng/mL	15.5	3.0	20.2%	15.5	4.1	26.6%
2.5 ng/mL	35.1	2.9	8.4%	35.1	3.5	10.0%
3.75 ng/mL	58.8	3.0	5.1%	58.8	3.8	6.5%
5 ng/mL	81.0	3.3	4.1%	81.0	3.7	4.6%
6.25 ng/mL	105.5	2.7	2.5%	105.5	3.9	3.7%
7.5 ng/mL	128.0	2.9	2.3%	128.0	3.5	2.8%
8.75 ng/mL	150.1	3.2	2.2%	150.1	4.2	2.8%
10 ng/mL	171.3	4.3	2.5%	171.3	5.2	3.0%

5 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
1.25 ng/mL	25 %	22	22 Neg	88	88 Neg
2.5 ng/mL	50 %	22	22 Neg	88	88 Neg
3.75 ng/mL	75 %	22	22 Neg	88	88 Neg
5 ng/mL	100 %	22	13 Neg/ 9 Pos	88	59 Neg/ 29 Pos
6.25 ng/mL	125 %	22	22 Pos	88	88 Pos
7.5 ng/mL	150 %	22	22 Pos	88	88 Pos
8.75 ng/mL	175 %	22	22 Pos	88	88 Pos
10 ng/mL	200 %	22	22 Pos	88	88 Pos

Semi-quantitative analysis: The following concentrations were determined with reference curve from 5 calibrators. Typical results were measured in ng/mL.

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.2	0.1	N/A	0.2	0.1	N/A
1.25 ng/mL	1.4	0.1	10.5%	1.4	0.2	12.6%
2.5 ng/mL	2.5	0.1	4.6%	2.5	0.2	6.5%
3.75 ng/mL	3.6	0.1	3.7%	3.6	0.2	5.0%
5 ng/mL	4.8	0.1	2.9%	4.8	0.2	3.9%
6.25 ng/mL	6.1	0.2	3.0%	6.1	0.2	3.4%
7.5 ng/mL	7.4	0.2	2.5%	7.4	0.2	3.3%
8.75 ng/mL	8.6	0.2	1.8%	8.6	0.2	2.6%
10 ng/mL	9.9	0.2	2.3%	9.9	0.4	3.6%

5 ng/mL Cutoff		Within Run (N=22)		Run-to-Run (N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
1.25 ng/mL	25 %	22	22 Neg	88	88 Neg
2.5 ng/mL	50 %	22	22 Neg	88	88 Neg
3.75 ng/mL	75 %	22	22 Neg	88	88 Neg
5 ng/mL	100 %	22	16 Neg/ 6 Pos	88	72 Neg/ 16 Pos
6.25 ng/mL	125 %	22	22 Pos	88	88 Pos
7.5 ng/mL	150 %	22	22 Pos	88	88 Pos
8.75 ng/mL	175 %	22	22 Pos	88	88 Pos
10 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy: One hundred (100) unaltered clinical urine specimens were tested with the LZI Fentanyl II (Semi-Quantitative) Enzyme Immunoassay and confirmed by LC/MS. Specimens having a norfentanyl concentration equal or greater than 5 ng/mL by LC/MS are defined as positive, and specimens with norfentanyl concentrations below 5 ng/mL by LC/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:

5 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
Positive	0	0	8**	10	40	100.0 %
Negative	20	20	2	0	0	84.0 %

The following table summarizes the result for the discordant samples:

5 ng/mL Cutoff	NFEN LC/MS (ng/mL)	LC/MS	LZI EIA (ng/mL)	LZI EIA
41**	2.7	-	6.6	+
43**	3.0	-	14.0	+
44**	3.0	-	6.5	+
45**	3.3	-	7.1	+
46**	3.5	-	10.2	+
47**	3.8	-	14.3	+
48**	3.9	-	6.9	+
49**	4.2	-	20.3	+

*** Discrepant between 50% below the cutoff concentration and cutoff (2.5 ng/mL – 4.9 ng/mL)*

Qualitative Accuracy Study:

5 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
Positive	0	1*	8**	10	40	100.0 %
Negative	20	19	2	0	0	82.0 %

The following table summarizes the results for the qualitative discordant samples:

Sample #	Norfentanyl LC/MS (ng/mL)	LC/MS Pos/Neg Result	AU480 EIA Qualitative Result (mAU)	AU480 EIA Qualitative Cutoff Rate (mAU)	LZI FEN II EIA Pos/Neg Result
37*	1.5	-	85.9	83.0	+
41**	2.7	-	111.3	83.0	+
43**	3.0	-	207.9	83.0	+
44**	3.0	-	107.7	83.0	+
45**	3.3	-	124.7	83.0	+
46**	3.5	-	169.6	83.0	+
47**	3.8	-	204.6	83.0	+
48**	3.9	-	113.6	83.0	+
49**	4.2	-	263.1	83.0	+

** Discrepant below 50% of the cutoff concentration (0 ng/mL – 2.49 ng/mL)*
*** Discrepant between 50% below the cutoff concentration and cutoff (2.5 ng/mL – 4.9 ng/mL)*

Discrepant samples contained levels of fentanyl that contributed to the false positive result.

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).

Fentanyl and Metabolites:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity	Result
Fentanyl	3.8	131.58 %	Positive
Norfentanyl	5	100.00 %	Positive

Structurally Related Compounds:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
4-Fluoro-Isobutyryl Fentanyl	20	25.00 %
9-Hydroxy Risperidone	100000	ND
Acetyl Fentanyl	7	71.43 %
Acetyl Norfentanyl	100	5.00 %
Acryl Fentanyl	4	125.00 %
Alfentanil	100000	ND
Butyryl Fentanyl	6	83.33 %
Butyryl Norfentanyl	40	12.50 %
Carfentanil Oxalate	100000	ND
Cis- d,I 3-Methyl Fentanyl	8	62.50 %
Cyclopropyl Fentanyl	3.2	156.25 %

Structurally Related Compounds, continued:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
Cyclopropyl Norfentanyl	25	20.00 %
Despropionyl Fentanyl (4-ANPP)	100000	ND
Furanyl Fentanyl	5.5	90.91 %
Furanyl Norfentanyl	180	2.78 %
(±) β-Hydroxy ThioFentanyl	5	100.00 %
Isobutyryl Fentanyl	15	33.33 %
Isobutyryl Norfentanyl	500	1.00 %
Labetalol Hydrochloride	100000	ND
Methoxyacetyl Fentanyl	3.5	142.86 %
MT-45	100000	ND
N-benzyl Furanyl Norfentanyl	11	45.45 %
N-benzyl Para-fluoro Norfentanyl	4	125.00 %
Norcarfentanil Oxalate	100000	ND
Ocfentanil	3.8	131.58 %
Para-fluoro Butyrl Fentanyl (p-FBF)	4.5	111.11 %
Para-fluoro Fentanyl	3.2	156.25 %
Remifentanil	100000	ND
Risperidone	100000	ND
Sufentanil	100000	ND
Thienyl Fentanyl	4	125.00 %
Thiofentanyl	3.2	156.25 %
Trans- d,I 3-Methyl Fentanyl	6	83.33 %
Trazodone	100000	ND
U-47700	100000	ND
Valeryl Fentanyl	70	7.14 %

Structurally Unrelated Compounds:

Compound	Spiked [ ] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Acetaminophen	100000	ND	Neg	Pos
6-Acetylmorphine	100000	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
d-Amphetamine	100000	ND	Neg	Pos
Atorvastatin	100000	ND	Neg	Pos
Benzoylecgonine	100000	ND	Neg	Pos
Buprenorphine	100000	ND	Neg	Pos
Bupropion	100000	ND	Neg	Pos
Caffeine	100000	ND	Neg	Pos
Carbamazepine	100000	ND	Neg	Pos
Cetirizine	100000	ND	Neg	Pos
Chlorpheniramine	100000	ND	Neg	Pos
Chlorpromazine	100000	ND	Neg	Pos
Clomipramine	100000	ND	Neg	Pos
Codeine	100000	ND	Neg	Pos
Desipramine	100000	ND	Neg	Pos
Dextromethorphan	40000	0.01 %	Pos	Pos
Diphenhydramine	100000	ND	Neg	Pos
Duloxetine	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
Ibuprofen	100000	ND	Neg	Pos
Imipramine	100000	ND	Neg	Pos
Lisinopril	100000	ND	Neg	Pos
Losartan	100000	ND	Neg	Pos
Loratadine	100000	ND	Neg	Pos
MDA (3,4-methylene-dioxyamphetamine)	100000	ND	Neg	Pos
MDEA	100000	ND	Neg	Pos
MDMA (3,4-methylene-dioxymethamphetamine)	100000	ND	Neg	Pos
Meperidine	100000	ND	Neg	Pos
Metformin	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
Nalmefene	100000	ND	Neg	Pos
Nicotine	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
(1S,2S)-(+)Pseudoephedrine	100000	ND	Neg	Pos
Quetiapine	100000	ND	Neg	Pos

Structurally Unrelated Compounds, continued:

Compound	Spiked [ ] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Ranitidine	100000	ND	Neg	Pos
Salbutamol (Albuterol)	100000	ND	Neg	Pos
Sertraline	100000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	100000	ND	Neg	Pos
l-Thyroxine	100000	ND	Neg	Pos
Tramadol	100000	ND	Neg	Pos
Zolpidem	100000	ND	Neg	Pos
Phencyclidine	100000	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.

The following structurally unrelated compound which showed interference at ±25 % of the cutoff concentration was then spiked into pooled negative human urine at ±50 % of cutoff concentrations (2.5 ng/mL and 7.5 ng/mL) for the assay. Interference was still observed with dextromethorphan. Results are summarized in the following table:

Compound	Spiked [ ] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	2.5 ng/mL	7.5 ng/mL
Dextromethorphan	40000	Pos	Pos	Pos

Endogenous and Preservative Compound Interference Study:

The following endogenous compounds were spiked into pooled negative human urine and the two levels of controls (3.75 ng/mL and 6.25 ng/mL) for the assay. The spiked solution was evaluated against cutoff calibrator. Interference was observed with Boric Acid. No other major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 5 ng/mL. Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Biotin	0.5	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride (CaCl2)	300	Neg	Neg	Pos
Citric Acid (pH 3)	200	Neg	Neg	Pos
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
Human Serum Albumin	500	Neg	Neg	Pos
Human Urine (pooled)	N/A	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	1000	Neg	Neg	Pos
Riboflavin	7.5	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	1000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
LZI Urine-Based Calibrator Buffer	N/A	Neg	Neg	Pos

The following endogenous compounds which showed interference at ±25 % of cutoff concentrations were then spiked into negative urine and at ±50 % of cutoff concentrations (2.5 ng/mL and 7.5 ng/mL) for the assay. Interference was still observed with Boric Acid at 1 % w/v. Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked Norfentanyl Concentration		
		0 ng/mL	2.5 ng/mL	7.5 ng/mL
Boric Acid	1000	Neg	Neg	Neg

pH Interference Study:

Negative urine and urine spiked with analyte to the two levels of controls (3.75 ng/mL and 6.25 ng/mL) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were evaluated against the cutoff calibrator.

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 5 ng/mL. Results are summarized in the following table:

pH Interference Study, continued:

pH	Spiked Norfentanyl Concentration		
	0 ng/mL	3.75 ng/mL Control	6.25 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 1.000 to 1.027 were split into three portions each and either left un-spiked or spiked to a norfentanyl concentration of either 3.75 or 6.25 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No interference was observed.

Specific Gravity	Spiked Norfentanyl Concentration		
	0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
1.000	Neg	Neg	Pos
1.003	Neg	Neg	Pos
1.005	Neg	Neg	Pos
1.008	Neg	Neg	Pos
1.010	Neg	Neg	Pos
1.012	Neg	Neg	Pos
1.015	Neg	Neg	Pos
1.018	Neg	Neg	Pos
1.020	Neg	Neg	Pos
1.022	Neg	Neg	Pos
1.025	Neg	Neg	Pos
1.027	Neg	Neg	Pos

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 2-8ºC 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. 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

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, **23**(82):7920-7970 (2017).
3. Ikeda R., Pelton C. Diversion programs for impaired physicians. *West J Med*, **152**: 617-621 (1990).
4. Poklis A., Fentanyl: a review for clinical and analytical toxicologists. Clinical Toxicology, 33, 439–447 (1995).

Bibliography, continued:

5. Jaffe J.H., Martin W.R., Opioid analgesics and antagonists. In: Pharrnacological Basis of Therapeutics, 7th Ed. Gilman AG, Goodman LS, Rall TW, Murad F, eds. New York: MacMillian, 517 (1985).
6. Ellenhorn M.D., Schonwald S., Ordog G., Wasserberger J, Fentanyl, in: Ellenhorns Medical Toxicology, Williams & Wilkins, Baltimore, Philadelphia, London, Paris, Bangkok, Buenos Aires, Hong Kong, Munich, Sydney, Tokyo, Wroclaw, 416–420 (1997).
7. Atluri S., Sudarshan G., Manchikanti L., Assessment of the trends in medical use and misuse of opioid analgesics from 2004 to 2011. *Pain Physician*, 17, E119–E128 (2014).
8. McClain DA, Hug CC Jr., Intravenous fentanyl kinetics. *Clin Pharmacol Ther* **28**:106-114 (1980).
9. Hug C.C., Murphy M.R., Anesthesiology, 55 (1981) 369. T. Tateishi, A.J.J. Wood, F.P. Guengerich and M. Wood, Biochem. Pharmacol., 50 (1995) 1921.
10. Baselt R.C., Disposition of Toxic Drugs and Chemicals in Man, 10th edition. Biomedical Publications: Seal Beach, California; 846–849 (2014).
11. Baum R.M., New variety of street drugs poses growing problem. *Chem. Eng News* Sep **9**:7-16 (1985).
12. Suzuki S., Inoue, T., New abused drug "China White". *Forensic Toxicol. News* **5**:5-12 (1987).
13. Heagy J.A., Identification of 4-propionoxy-4-phenyl-N-methylpiperidine. *Microgram* **15**: 181-186 (1982).
14. Poklis J., Poklis A., Wolf C., Hathaway C., Arbefeville E., Chrostowski L., Devers K., Hair L., Mainland M., Merves M., and Pearson J., *Journal of Analytical Toxicology*, **40**:703–708 (2016).
15. Backberg M., Beck O., Jonsson K.H., Helander A., Opioid intoxications involving butyryl fentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. *Clinical toxicology*, **53**:609–617 (2015).
16. EMCDDA. European Drug Report 2014: Trends and developments 2014. Available at: http://www.emcdda.europa.eu/attachements.cfm/att_228272_EN_TDAT14001ENN.pdf.
17. EMCDDA. (2015) New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015). Available at: http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf.
18. Helander A., Backberg M., Hulten P., Al-Saffer Y., Beck O., Detection of new psychoactive substance use among emergency room patients: results from Swedish STRIDA project. Forensic Science International, **243**;23–29(2014).
19. Rubenstein K.E., Schneider R.S., Ullman E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**: 846 (1972).
20. 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
21. Hammargren, W.R. and G.L. Henderson. Analyzing normetabolites of the Fentanyls by Gas Chromatography/Electron Capture Detection. *J. of Anal. Tox*. **12**:183-191 (1988).
22. Thevis, M., Geyer, H., Bahr, D., and W. Schänzer. Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. *Eur. J. Mass Spectrom*. **11**:419–427 (2005).
23. Gonzales, E., Ng, G., Pesce, A., West, C., West, R., Mikel, C., Latyshev, S., and P. Almazan. Stability of pain-related medications, metabolites, and illicit substances in urine. *Clinica Chimica Acta* **416**:80–85 (2013).

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.

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

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