

ARK™ Fentanyl II 0.5 ng/mL Assay

This ARK Diagnostics, Inc. package insert for the ARK Fentanyl II 0.5 ng/mL Assay must be read prior to use. Package insert instructions must be followed accordingly. The assay provides a simple and rapid analytical screening procedure for detecting Fentanyl in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

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KEY TO SYMBOLS USED

	Batch code	 YYYY-MM-DD	Use by/Expiration date
	Catalog Number		Manufacturer
	Consult Instructions for Use	 	Reagent 1/ Reagent 2
	Temperature limitation		<i>In Vitro</i> Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 NAME

ARK™ Fentanyl II 0.5 ng/mL Assay

2 INTENDED USE

The ARK Fentanyl II 0.5 ng/mL Assay is an immunoassay intended for the qualitative detection of fentanyl in human urine at a cutoff concentration of 0.5 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This in vitro diagnostic device is intended solely for use in employment and insurance testing.

The ARK Fentanyl II 0.5 ng/mL Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed positive analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

3 SUMMARY AND EXPLANATION OF THE TEST

Fentanyl [N-(1-(2-phenylethyl)-4-piperidyl)-N-phenylpropanamide] is a synthetic opioid narcotic analgesic similar to morphine.¹ Fentanyl is 50-100 times more potent than morphine. It is prescribed for patients with chronic pain and is used to manage pain after surgery or for treatment of breakthrough pain in cancer patients.² Fentanyl is prescribed in various forms: by injection (intravenous or intramuscular), transdermal patch³, and orally (transmucosal lozenge or film). Fentanyl such as the transdermal system can be abused in a manner similar to other opioid agonists, legal or illicit. All patients receiving opioids should be routinely monitored for signs of misuse, abuse and addiction.



Fentanyl has high potency and short duration of action, and it is abused for its intense euphoric effects. It is very dangerous when substituted illicitly for other opioids because of its potency and overdoses can lead to respiratory depression and death.^{4,5} It is a Schedule II substance under the U.S. Controlled Substances Act.

The determination of fentanyl in human urine aids the assessment of compliance for pain medication or for substance abuse. The ARK Fentanyl II 0.5 ng/mL Assay detects fentanyl in human urine. The test is not intended to differentiate between drugs of abuse and prescription use of fentanyl. There are no uniformly recognized drug levels for fentanyl in urine.

The primary metabolism of fentanyl leads to the time-dependent urinary excretion of fentanyl and norfentanyl.^{6,9} The half-life of fentanyl may range from 3 – 12 hours. Fentanyl is exclusively metabolized by N-dealkylation and hydroxylation. More than 90% of the dose is eliminated as norfentanyl and hydroxylated metabolites. Less than 7% of the dose is excreted unchanged in the urine.

4 PRINCIPLES OF THE PROCEDURE

The ARK Fentanyl II 0.5 ng/mL Assay is a homogeneous enzyme immunoassay technique used for the analysis of a specific compound in human urine. The assay is based on competition for antibody binding sites between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH). As the latter is bound by antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration in the sample. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

REF	Product Description	Quantity/Volume
5072-0001-00	ARK Fentanyl II 0.5 ng/mL Assay Reagent  – Antibody/Substrate Rabbit monoclonal antibodies to fentanyl, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 500 mL
	Reagent  – Enzyme Fentanyl derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 500 mL

5 REAGENTS

Reagent Handling and Storage

ARK Fentanyl II 0.5 ng/mL Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK Fentanyl products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use. For prescription use only. *Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.*
- Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Do not use reagents after the expiration date.
- Reagents contain ≤0.09% sodium azide.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human urine is required. Treat as potentially infectious material.
- Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical and physical integrity of the urine sample from the time it is collected until the time it is assayed, including during transport. Fresh urine specimens are suggested.
- Cap the urine sample immediately after collection, store refrigerated at 2–8°C (36–46°F) and assay within 7 days after collection. If the assay cannot be performed within 7 days, store the urine sample frozen at -20°C for up to 6 months prior to analysis.^{9,10,11,12}
- To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- The recommended pH range for urine specimens is 4.0 – 11.0¹³.
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

8 PROCEDURE

Materials Provided

ARK Fentanyl II 0.5 ng/mL Assay – **REF** 5072-0001-00

Materials Required – Provided Separately

ARK Fentanyl Negative Calibrator (0.0 ng/mL) – **REF** 5052-0002-01

ARK Fentanyl Cutoff Calibrator (0.5 ng/mL) – **REF** 5052-0002-02

Quality Controls – ARK Fentanyl Control (0.25 ng/mL and 0.75 ng/mL) – **REF** 5052-0003-00

Instruments

Reagents **R1** and **R2** may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of **R1** and **R2**. Refer to the instrument-specific operator's manual for daily maintenance. Consult the analyzer-specific application sheet for programming the fentanyl assay or contact Customer Support.

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual.

Qualitative Results

Use the 0.5 ng/mL Calibrator as a Cutoff Calibrator to distinguish negative and positive samples. Run the Low and High Controls as Negative and Positive respectively. Report test results less than the rate (mA/min) value for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate (mA/min) value for the Cutoff Calibrator as Positive.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Fentanyl II 0.5 ng/mL Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Each laboratory should establish its own ranges for each new lot of controls. Control results should fall within established ranges as determined by laboratory procedures and guidelines.

The ARK Fentanyl Control (0.25 ng/mL and 0.75 ng/mL) is intended for use in quality control of the ARK Fentanyl II 0.5 ng/mL Assay.

The Low Control should be Negative and the High Control should be Positive relative to the 0.5 ng/mL Cutoff Calibrator.

9 RESULTS AND EXPECTED VALUES

Qualitative Analysis - Negative Results

A specimen that gives a rate (mA/min) value less than the ARK Fentanyl II 0.5 ng/mL Cutoff Calibrator rate (mA/min) value is interpreted as negative; either the specimen does not contain fentanyl or fentanyl is present in a concentration below the cutoff level of this assay.

Qualitative Analysis - Positive Results

A specimen that gives a rate (mA/min) value equal to or greater than the ARK Fentanyl II 0.5 ng/mL Cutoff Calibrator rate (mA/min) value is interpreted as positive, indicating that fentanyl is present.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

10 LIMITATIONS

- The assay is designated for use with human urine only.
- ARK Fentanyl II 0.5 ng/mL Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Fentanyl II 0.5 ng/mL Assay indicates only the presence of fentanyl and does not necessarily correlate with the extent of physiological and psychological effects.
- Do not use Boric Acid as a preservative.**
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- It is possible that substances other than those tested in the specificity study may interfere with the test and cause false results.

11 SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were collected on the Beckman Coulter AU680[®] automated clinical chemistry analyzer using the ARK Fentanyl II 0.5 ng/mL Assay.

Precision

Precision was determined by assaying fentanyl in human urine. Drug-free, negative human urine was supplemented with fentanyl (0.00 to 1.00 ng/mL). Testing was performed for 20 days, 2 runs per day in quadruplicate (N=160).

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Results
0.00	-100	160	160 Negative
0.25	-50	160	160 Negative
0.38	-25	160	160 Negative
0.50	Cutoff	160	105 Negative; 55 Positive
0.62	+25	160	18 Negative; 142 Positive
0.75	+50	160	160 Positive
1.00	+100	160	160 Positive

Analytical Specificity

All compounds tested were added to drug-free, negative human urine.

The cross-reactivity of the following metabolites and structural analogs of fentanyl was evaluated by spiking these compounds into drug-free, negative human urine and evaluated by dose-response to determine the approximate equivalence to the 0.5 ng/mL fentanyl cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:

$\% \text{ Cross-reactivity} = (\text{Cutoff concentration} / \text{Concentration approximately equivalent to the } 0.5 \text{ ng/mL cutoff}) \times 100$

For the compounds Alfentanil and Remifentanil that did not produce a positive result, the highest concentration tested was used to calculate percent cross-reactivity.

Cross-reactivity

For the major metabolite, norfentanyl, the lowest concentration capable of producing a positive result is shown below.

Norfentanyl (Major Metabolite)

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
Norfentanyl	6.4	7.8

Other Metabolites and Structural Analogs of Fentanyl

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Percent Cross-reactivity (%)
Acetyl fentanyl	0.6	79.37
Furanyl fentanyl	0.6	87.72
ω-1-Hydroxyfentanyl	0.6	83.33
Isobutyryl fentanyl	0.6	79.37
Ocfentanil	0.8	65.79
Para-fluoro fentanyl	0.8	64.10
4-Fluoro-isobutyryl fentanyl	0.8	62.50
Acrylfentanyl	0.8	61.73
Butyryl fentanyl	0.8	60.98
Para-fluorobutyryl fentanyl (p-FBF)	0.9	55.56
Valeryl fentanyl	1.0	48.54
β-hydroxyfentanyl	4.0	12.41
Acetyl norfentanyl	6.1	8.17
(±) β-hydroxythiofentanyl	16.7	2.99
(±)-3-cis-methyl fentanyl	69.5	0.72
Despropionyl fentanyl (4-ANPP)	252.0	0.20
Carfentanil	389.6	0.13
Sufentanil	963.0	0.05
Norcarfentanil	1,130	0.04
Remifentanil	10,000	<0.005
Alfentanil	100,000	<0.001

The following compounds were negative at the concentrations tested.

Compound	Concentration Tested (µg/mL)	Compound	Concentration Tested (µg/mL)
(±)11-nor-9-carboxy-Δ9-THC	100	Methadone	100
6-Acetyl morphine	100	Methapyrilene	100
7-Hydroxymitragynine	50	Methaqualone	100
9-OH-Risperidone	100	Methylphenidate	100
Acetaminophen	500	Metoprolol	100
Acetylsalicylic acid	1000	Metronidazole	300
AH 7921	100	Mirtazepine	100
Albuterol (salbutamol)	100	Mitragynine	50
Alprazolam	100	Morphine	100
Amitriptyline	100	Morphine-3-glucuronide	100
Amlodipine	100	Naloxone	100
Amobarbital	100	Naltrexone	100
Amoxapine	100	Naproxen	100
Amphetamine	100	N-Desmethyloclozapine	100
Aripiprazole	100	N-Desmethyloanzapine	100
Benazepril	100	Nicotine	100
Benzoyllecgonine	100	Norbuprenorphine	100
Bisoprolol	100	Norcodeine	100
Buprenorphine	100	Norfenfluramine	100
Buprenorphine glucuronide	50	Norketamine	100
Bupropion	100	Nomeperidine	100
Caffeine	100	Normorphine	100
Cannabidiol	100	Noroxycodone	100
Carbamazepine	100	Norquetiapine	100
Carisoprodol	100	Olanzapine	100
Cetirizine	100	Omeprazole	100
Chlorpromazine	100	Oxazepam	100
Cimetidine	100	Oxycodone	100
Clomipramine	100	Oxymorphone	100
Codeine	100	Paliperidone	100
Cotinine	100	Paroxetine	100
Cyclobenzaprine	100	Pentazocine (Talwin)	100
Dehydroaripiprazole	100	Perphenazine	100
Desipramine	100	Phencyclidine	100
Dextromethorphan	100	Phenethylamine	100
Dihydrocodeine	100	Phenobarbital	100
Diltiazem	100	Promethazine	100
Diphenhydramine	100	Propoxyphene	100
D-Methamphetamine	100	Psilocin	100
DMT	100	Psilocybin	100
Doxepin	100	Quetiapine	100
Doxylamine	100	Quinidine	100
Ecgonine	100	Quinine	100
EDDP	100	Ranitidine	100
EMDP	100	Risperidone	100

Compound	Concentration Tested (µg/mL)	Compound	Concentration Tested (µg/mL)
Ephedrine	100	Ritalinic acid	100
Fexofenadine	100	Secobarbital	100
Fluoxetine	100	Sertindole	100
Fluphenazine	100	Sertraline	100
Furosemide	100	Sildenafil	100
Gabapentin	100	Tapentadol	100
Haloperidol	100	Thioridazine	100
Heroin	100	Tilidine	100
Hydrochlorothiazide	100	Tramadol	100
Hydrocodone	100	Tramadol-N-Desmethyl	100
Hydromorphone	100	Tramadol-O-Desmethyl	100
Hydroxyzine	100	Trazodone	100
Ibuprofen	500	Trimethoprim	100
Imipramine	100	Trimipramine	100
Ketamine	100	U-47700	100
Labetalol	100	U-48800	100
Levorphanol	100	U-49900	100
Levothyroxine	100	U-51754	100
Lidocaine	100	Valproic acid	250
Lisinopril	100	Venlafaxine	100
Loperamide	100	Verapamil	100
Loratadine	100	W-18	100
Maprotiline	100	Ziprasidone	100
mCPP	100	Zolpidem	100
Meperidine	100	Zopiclone	100
Metformin	100		

The following compounds were evaluated by dose-response to determine the approximate equivalence to the 0.5 ng/mL fentanyl cutoff.

Compound	Concentration Approximately Equivalent to the Cutoff (µg/mL)
Melperone	22.2
MDMA	37.5
Pipamperone	44.1
Buspiron	47.7
Nortriptyline	55.7
Protriptyline	62.7

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into fentanyl-spiked urine (± 50% of the cutoff concentration). No interference was observed when tested with the ARK Fentanyl II 0.5 ng/mL Assay.

Compound	Concentration Tested (mg/dL)	0.25 ng/mL (-50% Cutoff)	0.75 ng/mL (+50% Cutoff)
Acetone	1000	Negative	Positive
Ascorbic Acid	560	Negative	Positive
Bilirubin	2	Negative	Positive
Creatinine	500	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
Gamma Globulin	500	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	500	Negative	Positive
Human Albumin	500	Negative	Positive
Oxalic Acid	100	Negative	Positive
Riboflavin	7.5	Negative	Positive
Sodium Chloride	4000	Negative	Positive
Urea	2000	Negative	Positive

Interference – Boric Acid

One percent (1%) w/v of boric acid was added into fentanyl-spiked urine (± 50% of the cutoff concentration). Results are provided in the table below.

Compound	Conc. Tested	0.25 ng/mL (-50% Cutoff)	0.75 ng/mL (+50% Cutoff)
Boric Acid	1% w/v	Negative	Negative

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.002 to 1.030 g/mL and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of fentanyl at \pm 50% of the cutoff concentration. No interference was observed.

Comparative Analysis

Ninety-seven (97) confirmed fentanyl-positive and fifty (50) confirmed fentanyl-negative clinical urine specimens were analyzed by ARK Fentanyl II 0.5 ng/mL Assay. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory and used a fentanyl cutoff of 0.2 ng/mL. The ARK Fentanyl II 0.5 ng/mL Assay (cutoff 0.5 ng/mL) distinguished positive and negative results: overall agreement 100.0%, 100.0% specificity and 100.0% sensitivity.

		LC-MS/MS	
		(+)	(-)
ARK Fentanyl II 0.5 ng/mL Assay	(+)	97	0
	(-)	0	50

12 REFERENCES

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13 TRADEMARKS

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