DRI™ Fentanyl Assay

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For In Vitro Diagnostic Use

REF 10016437 (3x18 mL Kit)

Intended Use
The DRI™ Fentanyl Enzyme Immunoassay is intended for the qualitative determination of Fentanyl in human urine.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) and Liquid Chromatography/tandem mass spectrometry are the preferred confirmatory methods.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary and Explanation of the Test
Fentanyl is a synthetic opiate analgesic similar to morphine. Fentanyl is 50-100 times more potent than morphine. It is prescribed mainly for patients with chronic pain and is generally used to manage pain after surgery. Fentanyl is prescribed as intravenous anesthetic (Sublimaze®), transdermal patch (Duragesic®), and transmucosal Lozenge form (Actiq®). The fentanyl dose in the Duragesic ranges from 2.5-10 mg and in Actiq, it ranges from 0.2-1.6 mg. The half-life of Fentanyl is 3-12 hours.[2, 3] Fentanyl is exclusively metabolized by N-dealkylation and hydroxylation.[4, 5] More than 90% of the dose is eliminated as norfentanyl and hydroxylated metabolites.[6] Less than 7% of the dose is excreted unchanged in the urine.[7, 8]

The DRI Fentanyl Assay is supplied as liquid ready-to-use homogeneous enzyme immunoassay. The assay uses fentanyl specific monoclonal antibody that can detect fentanyl without any significant cross-reactivity to other opiate compounds.

The assay is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH), and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the conversion of nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents
REAGENT ***Antibody/Substrate Reagent (R1):***
Contains mouse monoclonal anti-fentanyl antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

REAGENT ***Enzyme Conjugate Reagent (R2):***
Contains fentanyl derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Materials Required (sold separately):

REF	Kit Description
1388	DRI Negative Calibrator (25 mL)
10016485	DRI Fentanyl 2 ng/mL Calibrator (10 mL)
10016484	DRI Fentanyl Low Control 1 ng/mL (25 mL)
10016486	DRI Fentanyl High Control 3 ng/mL (25 mL)

⚠ Precautions and Warning
DANGER: 1. The reagents are harmful if swallowed.
2. The reagents contain ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Mouse).
3. Reagents used in the assay components contain ≤0.09% sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing symptoms if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Reagent Preparation and Storage
The reagents are ready-to-use; no additional preparation is required. Reagents should be stored refrigerated. All assay components, opened or unopened, are stable until the expiration date indicated on their respective labels. Do not use the reagents beyond their expiration dates.

Specimen Collection and Handling
Collect urine specimens in plastic or glass containers. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Specimens kept at room temperature that do not receive initial test within 7 days of arrival at the laboratory should be placed into a secure refrigeration unit. Fentanyl is stable at 2-8°C and -20°C for up to 12 weeks.[9, 10, 11]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[12]

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. An effort should be made to keep pipetted samples free of gross debris. It is recommended that grossly turbid specimens be centrifuged before analysis. Frozen samples should be thawed and mixed prior to analysis. Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing. **Handle all urine specimens as if they were potentially infectious.**

Assay Procedure
Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this immunoassay. Refer to specific application instructions for each analyzer for chemistry parameters before performing the assay.

Quality Control and Calibration
Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Ensure that control results are within the established ranges, as determined by laboratory procedures and guidelines. If results fall outside of the established ranges, assay results are invalid. All QC requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values
The 2.0 ng/mL calibrator is used as a cutoff reference for distinguishing 'postive' from 'negative' samples. A sample that exhibits a change in absorbance values (ΔA) equal to or greater than that obtained with the cutoff calibrator is considered positive. A sample that exhibits a change in absorbance value (ΔA) lower than that obtained with the cutoff calibrator is considered as negative.

Limitations
1. A positive result from this assay indicates only the presence of fentanyl and does not necessarily correlate with the extent of physiological and psychological effects.
2. It is possible that other substances and/or factors (e.g.: technical or procedural), other than those investigated in the specificity study may interfere with the test and cause false results.

Specific Performance Characteristics
Typical performance results obtained on Beckman Coulter Olympus AU680 analyzer are shown below. The results obtained in your laboratory may differ from these data.

Precision

Fentanyl	n = 240	Within-run		Total run	
Cal / Ctrls	Mean (mA/min)	SD	CV%	SD	CV%
1 ng/mL	462	2.9	0.63	3.6	0.78
2 ng/mL	476	2.5	0.53	3.5	0.74
3 ng/mL	497	2.6	0.52	3.2	0.65

Interference

Endogenous and exogenous substances were tested for potential interference in the DRI Fentanyl Assay. No interference was observed in urine samples containing the compounds up to the concentrations listed below. Urine sample pH levels from 4 – 9 were also studied for possible interference.

Compound	Concentration (mg/dL)
Acetaminophen	10
Acetone	500
Acetyl Salicylic Acid	10
Ascorbic acid	150
Caffeine	10
Creatinine	400
Ethanol	1000
Galactose	5
Glucose	1000
Hemoglobin	150
Human Serum Albumin	200
Ibuprofen	10
Oxalic Acid	50
Riboflavin	3
Sodium chloride	400
Urea	1000
pH	4-9

Specificity

The cross-reactivity of Fentanyl, its metabolites, and analogs were evaluated by adding known amounts of each analyte to drug free urine. Results shown in the table below:

Fentanyl and its metabolites	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
Fentanyl	2	Pos	100
Despropionyl Fentanyl	100	Neg	<2
Nor-Fentanyl	10,000	Pos	0.02
Fentanyl Analogs			
Acetyl fentanyl	2	Pos	100
Acryl fentanyl	2	Pos	100
Alfentanil	10,000	Neg	<0.02
Benzyl fentanyl	4	Pos	50
β-Hydroxythiofentanyl	10	Pos	20
Butyryl fentanyl	1	Pos	200
Carfentanil	6	Pos	33.3
cis-3-methyl Fentanyl	150	Pos	1.3
Furanyl fentanyl	5	Pos	40
Isobutyryl fentanyl	2	Pos	100
meta-Fluorobutyryl fentanyl	3	Pos	66.7
Norcarfentanil	20,000	Pos	0.01
Ocfentanil	16	Pos	12.5
para-Fluorobutyryl fentanyl	2	Pos	100
para-Fluorofentanyl	2	Pos	100
para-methoxy Butyryl fentanyl	500	Pos	0.4
Remifentanil	2,500	Pos	0.08
Sufentanil	300	Pos	0.67
Thienyl fentanyl	16	Pos	12.5
trans-3-methyl Fentanyl	3	Pos	66.7
Valeryl fentanyl	2	Pos	100

Cross-reactivity of structurally related or unrelated opiate compounds were tested at the concentrations listed below

Structurally related or unrelated opiate compounds	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
6-Acetyl Morphine	50,000	Neg	<0.004
3,4 Methylenedioxy pyrovalerone	100,000	Neg	<0.002
7-Hydroxymitragynine	50,000	Neg	<0.004
AH 7921	10,000	Pos	0.02
Buprenorphine	100,000	Neg	<0.002
Buprenorphine glucuronide	50,000	Neg	<0.004
Codeine	100,000	Neg	<0.002
Dextromethorphan	200,000	Neg	<0.001
Dihydrocodeine	200,000	Neg	<0.001
EDDP	50,000	Neg	<0.004
Heroin	50,000	Neg	<0.004
Hydrocodone	100,000	Neg	<0.002
Hydromorphone	100,000	Neg	<0.002
Levorphanol	20,000	Neg	<0.01
Meperidine	100,000	Neg	<0.002
Methadone	150,000	Neg	<0.001
Mitragynine	100,000	Neg	<0.002
Morphine	200,000	Neg	<0.001
Morphine-3-Glucuronide	500,000	Neg	<0.001
Naloxone	25,000	Neg	<0.008
Naltrexone	25,000	Neg	<0.008
Norbuprenorphine	50,000	Neg	<0.004
Norcodeine	100,000	Neg	<0.002
Normeperidine	50,000	Neg	<0.004
Normorphine	100,000	Neg	<0.002
Oxycodone	200,000	Neg	<0.001
Oxymorphone	500,000	Neg	<0.001
Tapentadol	50,000	Neg	<0.004
Tapentadol-O-Beta-D-Glucuronide	50,000	Neg	<0.004
Thebaine	100,000	Neg	<0.002
Tilidine	50,000	Neg	<0.004
Tramadol	100,000	Neg	<0.002
Tramadol N-Desmethyl	100,000	Neg	<0.002
Tramadol O-Desmethyl	100,000	Neg	<0.002
U-47700	100,000	Pos	0.002
W-15	100,000	Neg	<0.002
W-18	100,000	Neg	<0.002

Cross-reactivity of structurally unrelated compounds were tested at the concentrations listed below

Structurally unrelated compounds	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
7-Aminoclonazepam	200,000	Neg	<0.001
7-Aminonitrazepam	200,000	Neg	<0.001
9-Hydroxyrisperidone	10,000	Pos	0.02
Acetaminophen	500,000	Neg	<0.001
Acetylsalicyclic acid	500,000	Neg	<0.001
Alprazolam	200,000	Neg	<0.001
Amitryptiline	25,000	Neg	<0.008
Amoxicillin	100,000	Neg	<0.002
Amphetamine	1,000,000	Neg	<0.001
Aripiprazole	500	Neg	<0.4
Benazepril	200,000	Neg	<0.001
Benzoylecgonine	1,000,000	Neg	<0.001
Bisprolol	200,000	Neg	<0.001
Bromazepam	200,000	Neg	<0.001
Buspirone	100,000	Neg	<0.002
Caffeine	100,000	Neg	<0.002
Carbamazepine	500,000	Neg	<0.001
Cetrizine	50,000	Neg	<0.004
Chlorazepam	200,000	Neg	<0.001
Chlordiazepoxide	200,000	Neg	<0.001
Chlorpromazine	100,000	Neg	<0.002
Cimetidine	500,000	Neg	<0.001
Clobazam	200,000	Neg	<0.001
Clomipramine	25,000	Neg	<0.008
Clonazepam	200,000	Neg	<0.001
Dehydroaripiprazole	500	Neg	<0.4
Delorazepam	200,000	Neg	<0.001
Desipramine	50,000	Neg	<0.004
Desmethylene Paroxetine	100,000	Neg	<0.002
Diazepam	200,000	Neg	<0.001
Diphenhydramine	10,000	Neg	<0.02
Diphenylmethoxyacetic acid	100,000	Neg	<0.002
Doxepine	50,000	Neg	<0.004
Doxylamine	100,000	Neg	<0.002
Ephedrine	250,000	Neg	<0.001
Fexofenadine	50,000	Neg	<0.004
Flunitrazepam	200,000	Neg	<0.001
Fluoxethine	100,000	Neg	<0.002
Fluphenazine	25,000	Neg	<0.008
Flurazepam	200,000	Neg	<0.001
Gabapentin	100,000	Neg	<0.002
Hydroxyzine	25,000	Neg	<0.008
Ibuprofen	500,000	Neg	<0.001
Imipramine	40,000	Neg	<0.005
Labetalol	100,000	Neg	<0.002
Loratidine	50,000	Neg	<0.004
Lorazepam	200,000	Neg	<0.001
Lormetazepam	200,000	Neg	<0.001
Maprotiline	500,000	Neg	<0.001
Medazepam	200,000	Neg	<0.001
Melperone	15,625	Pos	0.013

Table continued

Structurally unrelated compounds	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
Meta-Chlorophenyl piperazine	100,000	Neg	<0.002
Methylphenidate	100,000	Neg	<0.002
Metoprolol	200,000	Neg	<0.001
Metronidazole	200,000	Neg	<0.001
Mirtazapine	100,000	Neg	<0.002
Nalbuphine	500,000	Neg	<0.001
Naproxen	25,000	Neg	<0.008
Nitrazepam	200,000	Neg	<0.001
Nordiphenhydramine	100,000	Neg	<0.002
Norfenfluramine	10,000	Neg	<0.02
Nortriptyline	100,000	Neg	<0.002
Oxazepam	100,000	Neg	<0.002
Paroxetine maleate	100,000	Neg	<0.002
Phencyclidine	15,000	Neg	<0.013
Phenobarbital	500,000	Neg	<0.001
Pipamperone	15,625	Pos	0.013
Prazepam	200,000	Neg	<0.001
Pregabalin	100,000	Neg	<0.002
Promethazine	50,000	Neg	<0.004
Propoxyphene	15,000	Neg	<0.013
Quetiapine	100,000	Neg	<0.002
Ranitidine	200,000	Neg	<0.001
Risperidone	5,000	Pos	0.04
Ritalinic acid	100,000	Neg	<0.002
Ropinirole	50,000	Neg	<0.004
Secobarbital	500,000	Neg	<0.001
Talwin (Pentazocine)	10,000	Neg	<0.02
Temazepam	200,000	Neg	<0.001
Trazadone	10,000	Neg	<0.02
Triazolam	200,000	Neg	<0.001
Verapamil	10,000	Neg	<0.02
Zolpidem	100,000	Neg	<0.002
Zolpidem Phenyl-4-carboxylic acid	100,000	Neg	<0.002

Accuracy

A total of 170 clinical samples were tested in the DRI Fentanyl Assay in a qualitative mode and the results were compared to LC-MS/MS method.

		LC-MS/MS +	LC-MS/MS -
DRI Fentanyl Assay	+	102	0
	-	4*	64

* LC-MS/MS values ranged from 2.0 to 2.4 ng/mL.

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Glossary:

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