

ARK™ Ketamine Assay

This ARK Diagnostics, Inc. package insert for the ARK Ketamine 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 ketamine in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable.

Customer Service













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Key to Symbols Used

	Batch code	 YYYY-MM-DD	Use by/Expiration date
	Catalog Number		Manufacturer
	Authorized Representative		CE Mark with notified body number
	Consult Instructions for Use		Reagent 1 / Reagent 2
	Temperature limitation		In Vitro Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 Name

ARK™ Ketamine Assay

2 Intended Use

The ARK Ketamine Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of ketamine in human urine at a cutoff concentration of 50 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This *in vitro* diagnostic device is for prescription use only.

The semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK Ketamine 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 Test

Ketamine ((+/-)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone) is a synthetic, non-barbiturate and rapid-acting general anesthetic that is indicated for use in both human and veterinary surgical procedures.^{1,2}

Ketamine is a Schedule III substance under the United States Controlled Substances Act for its potential for abuse and risk of dependence. Ketamine is structurally and pharmacologically similar to phencyclidine (PCP), but is less potent, has a faster onset and shorter duration of action relative to PCP. Ketamine produces a variety of symptoms including, but not limited to anxiety, dysphoria, disorientation, insomnia, flashbacks, hallucinations, and psychotic episodes.^{1,3}

Following administration in humans, ketamine is *N*-demethylated by liver microsomal cytochrome P450 enzymes into norketamine, which is the major active metabolite that may contribute to the analgesic effect following ketamine administration. Norketamine is then dehydrogenated to produce dehydronorketamine. Urinary concentrations of ketamine, norketamine and dehydronorketamine have been detected in human urine specimens following ketamine use. Approximately 2% is excreted in urine as unchanged ketamine, 2% as norketamine, 16% as dehydronorketamine and the rest as conjugates of hydroxylated metabolites.⁴⁻¹¹

4 Principles of the Procedure

The ARK Ketamine Assay is a homogeneous enzyme immunoassay method used for the analysis of ketamine in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration. 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.

5 Reagents

REF	Product Description	Quantity/Volume
5056-0001-00	ARK Ketamine Assay Reagent [R1] – Antibody/Substrate rabbit antibodies to ketamine, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent [R2] – Enzyme Ketamine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 14 mL

REF	Product Description	Quantity/Volume
5056-0001-01	ARK Ketamine Assay Reagent [R1] – Antibody/Substrate rabbit antibodies to ketamine, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent [R2] – Enzyme Ketamine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 58 mL

Reagent Handling and Storage

ARK Ketamine 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 Ketamine 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.
- 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

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- 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.^{12,13}
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- The presence of bubbles or foam on specimens can lead to short sample delivery and erroneous results.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- Each laboratory should consult available literature and internal data regarding specimen stability. The recommended pH range for urine specimens is 4.0 – 11.0.¹⁴
- 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 Ketamine Assay – [REF] 5056-0001-00 or 5056-0001-01

Materials Required – Provided Separately

ARK Ketamine Calibrator – [REF] 5056-0002-00

ARK Ketamine Calibrator A (Negative) – [REF] 5056-0002-01

ARK Ketamine Calibrator B (Cutoff) – [REF] 5056-0002-02

Quality Controls – ARK Ketamine Control – [REF] 5056-0003-00

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**. Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Ketamine Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets bearing the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

Assay Sequence

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

Qualitative Results

Use the 50 ng/mL Calibrator B as a Cutoff Calibrator to distinguish negative and positive samples. Run the ARK Ketamine Low (25 ng/mL) and High (75 ng/mL) Controls as Negative and Positive respectively. Report test results less than the response value for the Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the Cutoff Calibrator as Positive.

Semiquantitative Results

Perform a 5-point calibration procedure; run calibrators in duplicate. Verify the calibration curve with the ARK Ketamine Low (25 ng/mL) and High (75 ng/mL) quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK Ketamine calibrator level (500 ng/mL) may be diluted in ARK Ketamine Calibrator A (Negative urine) and retested.

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
- A stored calibration curve was effective up to at least 25 days based on supporting data

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Ketamine 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 Ketamine Control is intended for use in quality control of the ARK Ketamine Assay.

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the 50 ng/mL Cutoff Calibrator.

9 Results and Expected Values

The actual ketamine concentration cannot be determined. A confirmatory method is required.

Qualitative Analysis – Negative Results

A specimen that gives a response value less than the ARK Ketamine Calibrator B Cutoff response value is interpreted as negative; either the specimen does not contain ketamine or ketamine is present in a concentration below the cutoff level of this assay.

Qualitative Analysis – Positive Results

A specimen that gives a response value equal to or greater than the ARK Ketamine Calibrator B Cutoff response value is interpreted as positive, indicating that ketamine is present.

Semiquantitative Analysis

Semiquantitative results for positive specimens enable the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semiquantitative results also permit the laboratory to establish quality control procedures and assess reproducibility. Specimens with sample results above the highest ARK Ketamine calibrator level (500 ng/mL) may be diluted in ARK Ketamine Calibrator A (Negative urine) and retested.

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 Ketamine Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Ketamine Assay indicates only the presence of ketamine and does not necessarily correlate with the extent of physiological and psychological effects.
- 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 Ketamine Assay.

Precision

Drug-free, negative human urine was supplemented with ketamine (0.0 to 100.0 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days

(N=160) and evaluated both qualitatively and semiquantitatively. Results are summarized in the tables below.

Qualitative Precision

Human Urine (ng/mL)	% Cutoff	# of Determinations	Qualitative Precision Results
0.0	-100	160	160 Negative
12.5	-75	160	160 Negative
25.0	-50	160	160 Negative
37.5	-25	160	160 Negative
50.0	Cutoff	160	88 Negative/ 72 Positive
62.5	+25	160	160 Positive
75.0	+50	160	160 Positive
87.5	+75	160	160 Positive
100.0	+100	160	160 Positive

Semiquantitative Precision

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	0.04	160 Negative
12.5	-75	160	11.70	160 Negative
25.0	-50	160	25.01	160 Negative
37.5	-25	160	36.63	160 Negative
50.0	Cutoff	160	50.32	83 Negative / 77 Positive
62.5	+25	160	63.30	160 Positive
75.0	+50	160	75.52	160 Positive
87.5	+75	160	87.34	160 Positive
100.0	+100	160	100.14	160 Positive

Analytical Recovery

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with ketamine (625.0 ng/mL) and dilutions were made proportionally with drug-free human urine. Ketamine concentrations ranged from 50.0 to 500.0 ng/mL. At each level, percentage recovery was calculated based on the mean concentration (N=6) compared to the expected concentration. Results are summarized in the table below.

Theoretical Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
50.0	52.2	104.4
100.0	102.7	102.7
200.0	193.4	96.7
300.0	274.2	91.4
400.0	408.9	102.2
500.0	511.6	102.3

Analytical Specificity

Structurally Related Compounds

The following structurally related compounds were added to drug-free, negative human urine and tested with the ARK Ketamine Assay. The results were evaluated both qualitatively and semiquantitatively. The ketamine metabolites, norketamine and dehydronorketamine, were positive when tested at 100 ng/mL. The ketamine analog, methoxetamine^{15,16}, was negative when tested at 100,000 ng/mL.

Compound	Concentration Tested (ng/mL)	ARK Immunoassay Result
Norketamine	100	Positive
Dehydronorketamine	100	Positive
Methoxetamine	100,000	Negative

Structurally Unrelated Compounds

The following structurally unrelated compounds were added to drug-free, negative human urine and tested with the ARK Ketamine Assay. The results were evaluated both qualitatively and semiquantitatively. The compounds at the concentrations listed below were negative when tested with the ARK Ketamine Assay.

Compound	Concentration Tested (ng/mL)
4-Bromo-2,5-Dimethoxyphenethylamine	100,000
6-Acetylcodeine	100,000
6-Acetylmorphine	100,000
6 β -Naltrexol	100,000
7-Aminoclonazepam	100,000
7-Aminoflunitrazepam	100,000
7-Aminonitrazepam	100,000
11-hydroxy-delta-9-THC	100,000
11-nor-9-carboxy-THC	500,000
Acetaminophen	500,000
Acetylsalicylic Acid	100,000
Alprazolam	100,000
Amitriptyline	100,000
Amobarbital	100,000
S-(+)-Amphetamine	500,000
Benzoyllecgonine	100,000
Benzylpiperazine	100,000
Bromazepam	100,000
Buprenorphine	100,000
Bupropion	100,000
Butabarbital	100,000
Butalbital	500,000
Caffeine	100,000
Cannabidiol	100,000

Compound	Concentration Tested (ng/mL)
Cannabinol	100,000
Carbamazepine	20,000
Carisoprodol	100,000
Chlordiazepoxide	100,000
Chlorpromazine	50,000
cis-Tramadol	100,000
Clobazam	100,000
Clomipramine	100,000
Clonazepam	100,000
Cocaine	100,000
Codeine	100,000
Cotinine	100,000
Cyclobenzaprine	25,000
Delta-9-THC	100,000
Demoxepam	100,000
Desalkylflurazepam	100,000
Desipramine	25,000
Dextromethorphan	100,000
Diazepam	100,000
Digoxin	100,000
Dihydrocodeine	100,000
Diphenhydramine	500,000
Doxepin	100,000
Doxylamine	100,000
Ecgonine	100,000
Ecgonine Methyl Ester	100,000
EDDP	100,000
1R,2S (-)-Ephedrine	100,000
1S,2R (+)-Ephedrine	100,000
Ethyl-β-D-glucuronide	100,000
Ethylmorphine	100,000
Fenfluramine (+)	100,000
Fenfluramine (-)	100,000
Fentanyl	100,000
Flunitrazepam	100,000
Fluoxetine	100,000
Flurazepam	100,000
Haloperidol	100,000
Heroin	100,000
Hexobarbital	100,000
Hydrocodone	100,000
Hydromorphone	100,000
Ibuprofen	500,000
Imipramine	25,000
Lamotrigine	100,000
Levorphanol	100,000
Lidocaine	100,000
Lorazepam	100,000
Lorazepam Glucuronide	100,000
Lormetazepam	50,000
LSD	100,000
Maprotiline	100,000

Compound	Concentration Tested (ng/mL)
(+)-MDA	100,000
MDEA	100,000
MDMA	100,000
Meperidine	100,000
Meprobamate	100,000
Methadone	100,000
S(+)-Methamphetamine	500,000
Methaqualone	10,000
Methylphenidate	100,000
Midazolam	100,000
Morphine	100,000
Morphine-3 β -D-glucuronide	100,000
Morphine-6 β -D-glucuronide	100,000
Nalorphine	50,000
Naloxone	100,000
Naltrexone	100,000
Naproxen	100,000
N-desmethylnaltrexone	100,000
Nicotine	100,000
Nitrazepam	100,000
Norbuprenorphine	50,000
Norcodeine	100,000
Nordiazepam	100,000
Normorphine	100,000
Norpropoxyphene	100,000
Norpseudoephedrine	100,000
Norsertaline	100,000
Nortriptyline	100,000
Oxazepam	100,000
Oxycodone	100,000
Oxymorphone	100,000
Paraxanthine	100,000
PCP	100,000
Pentazocine	100,000
Pentobarbital	100,000
Phenobarbital	100,000
Phentermine	100,000
Phenylephedrine	100,000
Phenylpropanolamine	100,000
Phenytoin	100,000
PMA	100,000
Prazepam	100,000
Propoxyphene	100,000
Propranolol	100,000
Protriptyline	25,000
R,R (-)-Pseudoephedrine	100,000
S,S (+)-Pseudoephedrine	100,000
Ranitidine	100,000
Methylphenidate Metabolite (Ritalinic Acid)	100,000
Salicylic Acid	100,000
Secobarbital	100,000

Compound	Concentration Tested (ng/mL)
Sertraline	50,000
Sufentanil Citrate	100,000
Temazepam	100,000
Theophylline	100,000
Thioridazine	100,000
Trazodone	100,000
Triazolam	100,000
Trifluoromethylphenylpiperazine	100,000
Trimipramine	25,000
Venlafaxine	100,000
Verapamil	100,000
Zolpidem Tartrate	100,000

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with ketamine ($\pm 50\%$ of the cutoff concentration). The results were evaluated both qualitatively and semiquantitatively. No interference was observed when tested with the ARK Ketamine Assay.

Compound	Concentration Tested	25 ng/mL (-50% Cutoff)	75 ng/mL (+50% Cutoff)
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin – Conjugated	2 mg/dL	Negative	Positive
Bilirubin – Unconjugated	2 mg/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Positive
Creatinine	500 mg/dL	Negative	Positive
Ethanol	1000 mg/dL	Negative	Positive
Galactose	10 mg/dL	Negative	Positive
Gamma Globulin	500 mg/dL	Negative	Positive
Glucose	2000 mg/dL	Negative	Positive
Hemoglobin	300 mg/dL	Negative	Positive
Human Albumin	500 mg/dL	Negative	Positive
Oxalic Acid	100 mg/dL	Negative	Positive
Riboflavin	7.5 mg/dL	Negative	Positive
Sodium Azide	1% w/v	Negative	Positive
Sodium Chloride	6000 mg/dL	Negative	Positive
Sodium Fluoride	1% w/v	Negative	Positive
Urea	6000 mg/dL	Negative	Positive

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of

ketamine at \pm 50% of the cutoff concentration. The results were evaluated both qualitatively and semiquantitatively. No interference was observed when tested with the ARK Ketamine Assay.

Method Comparison

A total of one hundred (100) unaltered clinical human urine specimens that are not individually identifiable were analyzed for ketamine with the ARK Ketamine Assay in both qualitative and semiquantitative modes and the results were compared to LC-MS/MS. Results are summarized in the table below.

		LC-MS/MS	
		(+)	(-)
ARK Ketamine Assay (50 ng/mL Cutoff)	(+)	50	1
	(-)	0	49

12 References

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13 Trademarks

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