

## ARK™ HS Benzodiazepine II Assay

This ARK Diagnostics, Inc. package insert for the ARK HS Benzodiazepine II 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 benzodiazepines 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
	Authorized Representative		CE Mark
	Consult Instructions for Use	 	Reagent 1 / Reagent 2
	Temperature limitation		In Vitro Diagnostic Medical Device
<b>Rx Only</b>	For Prescription Use Only		

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 Reagent Kit  5073-0001-00

 Reagent Kit  5073-0001-01

 Reagent Kit  5073-0001-02

## 1 Name

### **ARK™ HS Benzodiazepine II Assay**

## 2 Intended Use

The ARK HS Benzodiazepine II Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of benzodiazepines in human urine at cutoff concentrations of 100 ng/mL and 200 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 HS Benzodiazepine II 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

Benzodiazepines belong to a broad class of CNS-depressant drugs known as sedative-hypnotics.<sup>1</sup> They are prescribed as anxiolytics, sleeping agents, anticonvulsants, muscle relaxers, and also widely used for preanesthetic medication and to supplement, induce, and maintain anesthesia.<sup>1,2,3</sup>

Although widely prescribed, benzodiazepines are also abused.<sup>2-4</sup> Chronic benzodiazepine use can cause physical dependence, with withdrawal symptoms of insomnia, agitation, irritability, muscle tension, and, in more severe cases, hallucinations, psychosis, and seizures.<sup>1,2</sup>

The ARK HS Benzodiazepine II Assay is designed to detect a wide range of benzodiazepine compounds and metabolites, without the need for a glucuronidase pre-treatment.

## 4 Principles of the Procedure

The ARK HS Benzodiazepine II Assay is a homogeneous enzyme immunoassay technique used for the analysis of benzodiazepines in human urine. The assay is based on competition between benzodiazepines in the specimen and benzodiazepine labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of benzodiazepines from the specimen, enzyme

activity increases and is directly related to the benzodiazepines 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
5073-0001-00	<b>ARK HS Benzodiazepine II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to benzodiazepines, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	<b>Reagent [R2] – Enzyme</b> Benzodiazepine 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
5073-0001-01	<b>ARK HS Benzodiazepine II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to benzodiazepines, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	<b>Reagent [R2] – Enzyme</b> Benzodiazepine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 58 mL

REF	Product Description	Quantity/Volume
5073-0001-02	<b>ARK HS Benzodiazepine II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to benzodiazepines, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 500 mL
	<b>Reagent [R2] – Enzyme</b> Benzodiazepine derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 250 mL

### Reagent Handling and Storage

ARK HS Benzodiazepine II 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 HS Benzodiazepine II products contain  $\leq 0.09\%$  sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides.

## 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  $\leq 0.09\%$  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.<sup>5,6</sup>
- 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.
- Bubbles or foam on the sample can lead to short sample delivery and falsely low results.
- 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 HS Benzodiazepine II Assay – **REF** 5073-0001-00, 5073-0001-01 or 5073-0001-02

### Materials Required – Provided Separately

ARK HS Benzodiazepine II Calibrator (Set) – **REF** 5073-0002-00

ARK HS Benzodiazepine II Calibrator A (Negative) – **REF** 5073-0002-01

ARK HS Benzodiazepine II Calibrator B (100 ng/mL Cutoff) – **REF** 5073-0002-02

ARK HS Benzodiazepine II Calibrator C (200 ng/mL Cutoff) – **REF** 5073-0002-03

ARK HS Benzodiazepine II Control (75 / 125 ng/mL) – REF 5073-0003-00

ARK HS Benzodiazepine II Control (150 / 250 ng/mL) – REF 5073-0003-01

### **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 ARK HS Benzodiazepine II Assay or contact Customer Support.

### **Assay Sequence**

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

### **Qualitative Results**

Use the 100 ng/mL Calibrator B or the 200 ng/mL Calibrator C as a Cutoff Calibrator to distinguish negative and positive samples. Run the ARK HS Benzodiazepine II Low (75 ng/mL) and High (125 ng/mL) Controls with Cutoff Calibrator B or the ARK HS Benzodiazepine II Low (150 ng/mL) and High (250 ng/mL) Controls with Cutoff Calibrator C, as Negative and Positive respectively. Report test results less than the response value for the applicable Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the applicable Cutoff Calibrator as Positive.

### **Semiquantitative Results**

Perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with the ARK HS Benzodiazepine II Low and High quality controls for your cutoff, according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK HS Benzodiazepine II calibrator level (3000 ng/mL) may be diluted in ARK HS Benzodiazepine II 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 15 days based on supporting data

### **Quality Control (QC) and Calibration**

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

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the applicable Cutoff Calibrator (100 ng/mL or 200 ng/mL).

## 9 Results and Expected Values

A more specific confirmatory method, such as LC-MS/MS or GC-MS, is required in order to obtain a confirmed positive result.

### Qualitative Analysis – Negative Results

A specimen that gives a response value less than the ARK HS Benzodiazepine II Calibrator B or ARK HS Benzodiazepine II Calibrator C Cutoff response value is interpreted as negative; either the specimen does not contain benzodiazepines or benzodiazepines are present in concentrations below the applicable cutoff level of this assay.

### Qualitative Analysis – Positive Results

A specimen that gives a response value equal to or greater than the ARK HS Benzodiazepine II Calibrator B or ARK HS Benzodiazepine II Calibrator C Cutoff response value is interpreted as positive, indicating that benzodiazepines are present.

### Semiquantitative Analysis

The actual Benzodiazepine concentration cannot be determined with this assay. 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 HS Benzodiazepine II calibrator level (3000 ng/mL) may be diluted in ARK HS Benzodiazepine II 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, particularly when the preliminary result is positive.

## 10 Limitations

- The assay is designated for use with human urine only.
- ARK HS Benzodiazepine II Assay reagents, calibrators and controls were developed to be used together. Performance with substituted products cannot be assured.
- A positive result using the ARK HS Benzodiazepine II Assay indicates only the presence of Benzodiazepines 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 HS Benzodiazepine II Assay.

### Precision

Drug-free, negative human urine was supplemented with Etizolam (0.0 to 200.0 ng/mL for the 100 ng/mL cutoff and 0.0 to 400.0 ng/mL for the 200 ng/mL cutoff). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated qualitatively and semi quantitatively. Results are summarized in the tables below.

#### Qualitative Precision – 100 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.0	-100	160	160 Negative
25.0	-75	160	160 Negative
50.0	-50	160	160 Negative
75.0	-25	160	160 Negative
100.0	Cutoff	160	104 Negative; 56 Positive
125.0	+25	160	160 Positive
150.0	+50	160	160 Positive
175.0	+75	160	160 Positive
200.0	+100	160	160 Positive

#### Semiquantitative Precision – 100 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	1.1	160 Negative
25.0	-75	160	23.2	160 Negative
50.0	-50	160	50.5	160 Negative
75.0	-25	160	74.0	160 Negative
100.0	Cutoff	160	98.6	93 Negative; 67 Positive
125.0	+25	160	123.3	160 Positive
150.0	+50	160	145.2	160 Positive
175.0	+75	160	170.5	160 Positive
200.0	+100	160	187.6	160 Positive

**Qualitative Precision – 200 ng/mL Cutoff**

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Qualitative Precision Results
0.0	-100	160	160 Negative
50.0	-75	160	160 Negative
100.0	-50	160	160 Negative
150.0	-25	160	160 Negative
200.0	Cutoff	160	142 Negative; 18 Positive
250.0	+25	160	160 Positive
300.0	+50	160	160 Positive
350.0	+75	160	160 Positive
400.0	+100	160	160 Positive

**Semiquantitative Precision – 200 ng/mL Cutoff**

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	1.1	160 Negative
50.0	-75	160	50.5	160 Negative
100.0	-50	160	98.6	160 Negative
150.0	-25	160	145.2	160 Negative
200.0	Cutoff	160	187.6	147 Negative; 13 Positive
250.0	+25	160	240.3	160 Positive
300.0	+50	160	293.2	160 Positive
350.0	+75	160	342.8	160 Positive
400.0	+100	160	391.1	160 Positive

**Analytical Recovery**

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with Etizolam (3750.0 ng/mL) and dilutions were made proportionally with drug-free human urine. Etizolam concentrations ranged from 25.0 to 3000.0 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 (%)
25.0	23.8	95.1
50.0	54.8	109.5
100.0	99.6	99.6
250.0	242.2	96.9
500.0	481.4	96.3
1000.0	951.9	95.2
1500.0	1422.4	94.8
2000.0	1929.9	96.5
2500.0	2333.7	93.3
3000.0	3107.7	103.6

### Analytical Specificity

All compounds tested were added to drug-free, negative human urine and tested with the ARK HS Benzodiazepine II Assay in both qualitative and semiquantitative modes.

The cross-reactivity of the following structurally related compounds was evaluated by spiking these compounds into drug-free, negative human urine to determine the minimum concentration that would give a positive result approximately equivalent to the 100 ng/mL or 200 ng/mL Etizolam cutoffs. These concentrations were used to determine the percent cross-reactivity according to the formula:

$$\% \text{ Cross-reactivity} = (\text{Cutoff concentration} / \text{Lowest concentration of cross-reactant causing a positive result}) \times 100$$

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

Structurally Related Compounds

<b>Compounds</b>	<b>Concentration Approximately Equivalent to 100 Cutoff (ng/mL)</b>	<b>Cross-reactivity at 100 Cutoff (%)</b>	<b>Concentration Approximately Equivalent to 200 Cutoff (ng/mL)</b>	<b>Cross-reactivity at 200 Cutoff (%)</b>
α-OH-alprazolam	55	181.8	100	200.0
α-OH-midazolam	11	909.1	16	1250.0
α-OH-Triazolam	20	500.0	35	571.4
2-OH-ethylflurazepam	70	142.9	125	160.0
3-OH-flubromazepam	19	526.3	27	740.7
3-OH-phenazepam	15	666.7	22	909.1
4-OH-alprazolam	110	90.9	190	105.3
7-aminoclonazepam	40	250.0	75	266.7
7-aminoflunitrazepam	150	66.7	270	74.1
7-aminonimetazepam	600	16.7	1200	16.7
7-aminonitrazepam	400	25.0	800	25.0
alprazolam	80	125.0	130	153.8
Bromazepam	140	71.4	270	74.1
Chlordiazepoxide	55	181.8	100	200.0
Clobazam	180	55.6	400	50.0
Clonazepam	100	100.0	180	111.1
Delorazepam	10	1000.0	15	1333.3
Demoxepam	100	100.0	180	111.1
Desalkylflurazepam	15	666.7	25	800.0
Clonazolam	350	28.6	800	25.0
Clorazepate	45	222.2	70	285.7
Deschloroetizolam	450	22.2	900	22.2
Diazepam	65	153.8	100	200.0
Diclazepam	15	666.7	25	800.0
Estazolam	60	166.7	85	235.3
Flualprazolam	28	357.1	45	444.4
Flubromazepam	25	400.0	38	526.3
Flubromazolam	40	250.0	68	294.1
Flunitrazepam	450	22.2	850	23.5
Flurazepam	350	28.6	650	30.8
Halazepam	2,500	4.0	5,000	4.0
Ketazolam	2,200	4.5	4,000	5.0
Loprazolam	130	76.9	260	76.9
Lorazepam	12	833.3	16.5	1212.1
Lorazepam glucuronide	13	769.2	20	1000.0
Lormetazepam	17	588.2	23	869.6

Compounds	Concentration Approximately Equivalent to 100 Cutoff (ng/mL)	Cross-reactivity at 100 Cutoff (%)	Concentration Approximately Equivalent to 200 Cutoff (ng/mL)	Cross-reactivity at 200 Cutoff (%)
Meclonazepam	36	277.8	57	350.9
Medazepam	250	40.0	500	40.0
Midazolam	16	625.0	25	800.0
N-Desmethyloclobazam	160	62.5	320	62.5
N-desmethyflunitrazepam	150	66.7	300	66.7
Nimetazepam	1,350	7.4	3,000	6.7
Nitrazepam	500	20.0	950	21.1
Norchlorodiazepoxide	75	133.3	120	166.7
Nordiazepam	43	232.6	57	350.9
Oxazepam	38	263.2	55	363.6
Oxazepam Glucuronide	20	500.0	30	666.7
Phenazepam	15	666.7	20	1000.0
Prazepam	1,700	5.9	3,400	5.9
Pyrazolam	350	28.6	700	28.6
Temazepam	85	117.6	130	153.8
Temazepam glucuronide	35	285.7	70	285.7
Tetrazepam	600	16.7	1,200	16.7
Triazolam	27	370.4	45	444.4

## Interference

### Structurally Unrelated Compounds

High concentrations of the following structurally unrelated compounds were spiked into drug-free urine and tested in semi-quantitative mode of analysis. The concentrations that corresponded to 100 ng/mL and 200 ng/mL of etizolam were identified and are shown in the following table. Compounds that failed to produce a positive response are shown as greater than (>) the highest concentration tested. When spiked into urine containing etizolam at the high control concentration (125 ng/mL for the 100 ng/mL cutoff, or 250 ng/mL for the 200 ng/mL cutoff), none of these compounds produced a negative result.

Compound	Compound concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)	Compound concentration approximately equivalent to 200 ng/mL Cutoff (ng/mL)
4-Hydroxydiclofenac	>100,000	>100,000
6-Acetylcodeine	>100,000	>100,000

<b>Compound</b>	<b>Compound concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)</b>	<b>Compound concentration approximately equivalent to 200 ng/mL Cutoff (ng/mL)</b>
6-Acetylmorphine	>100,000	>100,000
Acetaminophen	>100,000	>100,000
Acetylsalicylic Acid	>500,000	>500,000
Amitriptyline	>100,000	>100,000
Amlodipine	>100,000	>100,000
Amobarbital	>100,000	>100,000
S-(+) Amphetamine	>100,000	>100,000
Aripiprazole	>100,000	>100,000
Benzoylcegonine	>100,000	>100,000
Bisoprolol	>100,000	>100,000
4-Bromo-2,5-Dimethoxyphenethylamine	>100,000	>100,000
Buprenorphine	>100,000	>100,000
Bupropion	>100,000	>100,000
Buspirone	>100,000	>100,000
Butabarbital	>100,000	>100,000
Caffeine	>500,000	>500,000
Cannabidiol	>100,000	>100,000
Cannabinol	>100,000	>100,000
Carbamazepine	>100,000	>100,000
Carisoprodol	>100,000	>100,000
Chlorpromazine	>100,000	>100,000
Chlorprothixene	>100,000	>100,000
Cis-Tramadol	>100,000	>100,000
Clomipramine	>100,000	>100,000
Citalopram	>100,000	>100,000
Cocaine	>100,000	>100,000
Codeine	>100,000	>100,000
Cotinine	>100,000	>100,000
Cyclobenzaprine	>100,000	>100,000
Delta-9-THC	>100,000	>100,000
Desipramine	>100,000	>100,000
Dextromethorphan	>100,000	>100,000
Diclofenac	10,000	20,000
Dihydrocodeine	>100,000	>100,000
Diphenhydramine	>100,000	>100,000
Doxepin	>100,000	>100,000
Ecgonine	>100,000	>100,000

<b>Compound</b>	<b>Compound concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)</b>	<b>Compound concentration approximately equivalent to 200 ng/mL Cutoff (ng/mL)</b>
Ecgonine methyl ester	>100,000	>100,000
EDDP	>100,000	>100,000
1R, 2S(-)-Ephedrine	>100,000	>100,000
1S, 2R(+)-Ephedrine	>100,000	>100,000
Escitalopram	>100,000	>100,000
Ethyl β-D-glucuronide	>100,000	>100,000
Ethylmorphine	>100,000	>100,000
Fenfluramine	>100,000	>100,000
Fentanyl	>100,000	>100,000
Fluoxetine	>100,000	>100,000
Fluvoxamine	>100,000	>100,000
Gabapentin	>100,000	>100,000
Heroin	>100,000	>100,000
Hexobarbital	>100,000	>100,000
Hydrocodone	>100,000	>100,000
Hydromorphone	>100,000	>100,000
11-hydroxy-delta-9-THC	>100,000	>100,000
Ibuprofen	>100,000	>100,000
Imipramine	>100,000	>100,000
Indometacin	>100,000	>100,000
Ketamine	>100,000	>100,000
Lamotrigine	>100,000	>100,000
Levorphanol Tartrate	>100,000	>100,000
Lidocaine	>100,000	>100,000
Loperamide	>100,000	>100,000
LSD	>100,000	>100,000
Maprotiline	>100,000	>100,000
MDA	>100,000	>100,000
MDEA	>100,000	>100,000
MDMA	>100,000	>100,000
Meperidine	>100,000	>100,000
Meprobamate	>100,000	>100,000
Metamizole	>100,000	>100,000
Metformin	>100,000	>100,000
Methadone	>100,000	>100,000
S(+)-methamphetamine	>100,000	>100,000
Methaqualone	>100,000	>100,000
Methylphenidate	>100,000	>100,000

<b>Compound</b>	<b>Compound concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)</b>	<b>Compound concentration approximately equivalent to 200 ng/mL Cutoff (ng/mL)</b>
Metoclopramide	>100,000	>100,000
Metoprolol	>100,000	>100,000
Mirtazapine	>100,000	>100,000
Morphine	>100,000	>100,000
Morphine-3-beta-glucuronide	>100,000	>100,000
Morphine-6-beta-glucuronide	>100,000	>100,000
Nalorphine	>100,000	>100,000
Naloxone	>100,000	>100,000
Naltrexone	>100,000	>100,000
Naproxen	>100,000	>100,000
Nefopam	>100,000	>100,000
N-desmethyltapentadol	>100,000	>100,000
Norbuprenorphine	>100,000	>100,000
Norcodeine	>100,000	>100,000
Normorphine	>100,000	>100,000
Norpropoxyphene	>100,000	>100,000
Norpseudoephedrine	>100,000	>100,000
Nortriptyline	>100,000	>100,000
Olanzapine	>100,000	>100,000
Omeprazole	>100,000	>100,000
Opipramol	>100,000	>100,000
Oxaprozin	13,000	25,000
Oxycodone	>100,000	>100,000
Oxymorphone	>100,000	>100,000
Paraxanthine	>100,000	>100,000
Paroxetine	>100,000	>100,000
PCP	>100,000	>100,000
Pentazocine	>100,000	>100,000
Pentobarbital	>100,000	>100,000
Phenobarbital	>100,000	>100,000
Phentermine	>100,000	>100,000
Phenylephrine	>100,000	>100,000
Phenylpropanolamine	>100,000	>100,000
Phenytoin	>100,000	>100,000
PMA	>100,000	>100,000
Promethazine	>100,000	>100,000
Propoxyphene	>100,000	>100,000
Propranolol	>100,000	>100,000

<b>Compound</b>	<b>Compound concentration approximately equivalent to 100 ng/mL Cutoff (ng/mL)</b>	<b>Compound concentration approximately equivalent to 200 ng/mL Cutoff (ng/mL)</b>
Prothipendyl	>100,000	>100,000
Protriptyline	>100,000	>100,000
R,R(-)-Pseudoephedrine	>100,000	>100,000
S,S(+)-Pseudoephedrine	>100,000	>100,000
Quetiapine	>100,000	>100,000
Ramipril	>100,000	>100,000
Ranitidine	>100,000	>100,000
Ritalinic Acid	>100,000	>100,000
Salicylic Acid	>100,000	>100,000
Secobarbital	>100,000	>100,000
Sertraline	>100,000	>100,000
Sufentanil Citrate	>100,000	>100,000
11-nor-9-carboxy THC	>100,000	>100,000
Theophylline	>100,000	>100,000
Thioridazine	>100,000	>100,000
Tianeptine	>100,000	>100,000
Trifluoromethylphenylpiperazine	>100,000	>100,000
Trazodone	>100,000	>100,000
Trimipramine	>100,000	>100,000
Valproic Acid	>100,000	>100,000
Venlafaxine	>100,000	>100,000
Vortioxetine	>100,000	>100,000
Zaleplon	>100,000	>100,000
Zolpidem Tartrate	>100,000	>100,000
Zopiclone	>100,000	>100,000

### Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with Etizolam at  $\pm 25\%$  of the cutoff concentrations (75 ng/mL and 125 ng/mL for the 100 ng/mL cutoff, 150 ng/mL and 250 ng/mL for the 200 ng/mL cutoff). Only interference with Boric Acid was observed when tested with the ARK HS Benzodiazepine II Assay in both qualitative and semiquantitative modes.

<b>Substance</b>	<b>Concentration Tested</b>	<b>Spiked Etizolam Level</b>	
		<b>-25% Cutoff</b>	<b>+25% Cutoff</b>
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin	2 mg/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Negative

Substance	Concentration Tested	Spiked Etizolam Level	
		-25% Cutoff	+25% Cutoff
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	115 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

### Specific Gravity and pH

Urine samples with specific gravity values ranging 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 etizolam at  $\pm 25\%$  of the cutoff concentrations (75 ng/mL and 125 ng/mL for the 100 ng/mL cutoff, 150 ng/mL and 250 ng/mL for the 200 ng/mL cutoff). No interference was observed when tested with the ARK HS Benzodiazepine II Assay in both qualitative and semiquantitative modes.



## Method Comparison

A total of one hundred sixty-three (163) unaltered, un-pretreated with glucuronidase, clinical human urine specimens that are not individually identifiable were analyzed for benzodiazepine at the two cutoff levels with the ARK HS Benzodiazepine II Assay in semiquantitative modes and the results were compared to LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory. Briefly, the method involves treating specimens with glucuronidase, adding internal standards, and injecting into a column for LC-MS/MS. Detection peaks and their limits of quantitation (LoQ) in ng/mL are: 7-aminoclonazepam (5); alprazolam (1); hydroxyalprazolam (1); lorazepam (10); diazepam (5); nordiazepam (5); oxazepam (5); temazepam (1); midazolam (1); hydroxymidazolam (1). Results are summarized in the tables below, where the LC-MS/MS result represents the sum across all benzodiazepine peaks identified.

All ARK-positive samples in this study were confirmed by LC-MS/MS to have benzodiazepine concentrations of at least 20 ng/mL.

### Method Comparison – 100 ng/mL Cutoff

ARK Assay Result	LC-MS/MS Result (ng/mL)			
	Less than 50% of cutoff (< 50 ng/mL)	Between 50% below the Cutoff and the Cutoff (50 – 99.9 ng/mL)	Between the Cutoff and 50% above the Cutoff (100 – 149.9 ng/mL)	Equal or Greater than 50% above the Cutoff (≥ 150 ng/mL)
Negative (< 100 ng/mL)	71	3	2	1*
Positive (≥ 100 ng/mL)	8**	9	9	60

Sample ID	ARK Assay (ng/mL)	LC-MS/MS (ng/mL)	Benzodiazepines present by LC-MS/MS
62*	96.3	216.9	Alprazolam; hydroxyalprazolam
8**	118.2	39.1	7-aminoclonazepam
30**	661.8	26.4	Lorazepam
41**	183.8	23.9	Nordiazepam, oxazepam, temazepam
42**	466.3	27.1	Nordiazepam, oxazepam, temazepam
43**	520.1	23.5	Nordiazepam, oxazepam, temazepam
90**	> 3,000	46.0	Lorazepam
91**	192.3	28.4	Lorazepam

Sample ID	ARK Assay (ng/mL)	LC-MS/MS (ng/mL)	Benzodiazepines present by LC-MS/MS
93**	340.6	29.6	Lorazepam

\*\*Strong reactivity to 7-aminoclonazepam, diazepam, lorazepam-glucuronide, oxazepam-glucuronide and temazepam-glucuronide contributed to the positive results obtained with the ARK HS Benzodiazepine II Assay.

Method Comparison – 200 ng/mL Cutoff

ARK Assay Result	LC-MS/MS Result (ng/mL)			
	Below 50% of the cutoff (<100 ng/mL)	Between 50% below the Cutoff and the Cutoff (100 – 199.9 ng/mL)	Between the Cutoff and 50% above the Cutoff (200 – 299.9 ng/mL)	Equal or Greater than 50% above the Cutoff (≥ 300 ng/mL)
Negative (< 200 ng/mL)	78	6	2	2 <sup>†</sup>
Positive (≥ 200 ng/mL)	13 <sup>††</sup>	11	11	40

Sample ID	ARK Assay (ng/mL)	LC-MS/MS (ng/mL)	Benzodiazepines present by LC-MS/MS
55 <sup>†</sup>	180.9	386.1	Alprazolam; hydroxyalprazolam
61 <sup>†</sup>	176.6	543.4	Alprazolam; hydroxyalprazolam
30 <sup>††</sup>	661.8	26.5	Lorazepam
35 <sup>††</sup>	1,620.1	90.3	Lorazepam
42 <sup>††</sup>	466.3	27.1	Diazepam, nordiazepam, oxazepam, temazepam
43 <sup>††</sup>	520.1	23.5	Nordiazepam, oxazepam, temazepam
47 <sup>††</sup>	1,291.9	66.3	Nordiazepam, oxazepam, temazepam
48 <sup>††</sup>	838.8	66.5	Nordiazepam, oxazepam, temazepam
72 <sup>††</sup>	1,117.9	75.4	Nordiazepam, oxazepam, temazepam
82 <sup>††</sup>	811.7	73.0	Nordiazepam, oxazepam, temazepam
90 <sup>††</sup>	> 3,000	46.0	Lorazepam
93 <sup>††</sup>	340.6	29.6	Lorazepam
94 <sup>††</sup>	1,124.9	60.6	Lorazepam
96 <sup>††</sup>	697.6	57.5	Lorazepam
97 <sup>††</sup>	815.2	68.0	Lorazepam

††Strong reactivity with diazepam, lorazepam-glucuronide, oxazepam-glucuronide and temazepam-glucuronide contributed to the positive results obtained with the ARK HS Benzodiazepine II Assay.

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## 13 Trademarks

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