

## ARK™ Ethyl Glucuronide II Assay

This ARK Diagnostics, Inc. package insert for the ARK Ethyl Glucuronide 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 ethyl glucuronide 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













48089 Fremont Blvd  
 Fremont, CA 94538 USA  
 Tel: 1-877-869-2320  
 Fax: 1-510-270-6298  
 customersupport@ark-tdm.com  
 www.ark-tdm.com  
 SRN: US-MF-000023925







EC REP

Emergo Europe  
 Westervoortsedijk 60  
 6827 AT Arnhem  
 The Netherlands

### 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
<b>Rx Only</b>	For Prescription Use Only		

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Reagent Kit  5077-0001-00  
 Reagent Kit  5077-0001-01  
 Reagent Kit  5077-0001-02  
 Reagent Kit  5077-0001-03

## 1 Name

### **ARK™ Ethyl Glucuronide II Assay**

## 2 Intended Use

The ARK Ethyl Glucuronide II Assay is intended for the qualitative detection and/or semi-quantitative estimation of ethyl glucuronide in human urine at cutoff concentrations of 500 ng/mL and 1000 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers.

The semi-quantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution for the specimen for confirmation by a confirmatory method, or (2) permitting laboratories to establish quality control procedures.

The ARK Ethyl Glucuronide II Assay provides only a preliminary analytical 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

Assessment of ethanol consumption is important for medical treatment of persons addicted to alcohol. Forensic and work place applications are also common. Ethyl Glucuronide (EtG) is a direct metabolite of ethanol, which is formed by enzymatic conjugation of ethanol with glucuronic acid.<sup>1,2</sup> The metabolism of ethanol leads to the time-dependent urinary excretion of ethyl glucuronide and other metabolites. Alcohol in urine is normally detected for only a few hours, whereas ethyl glucuronide can be detected up to several days even after complete elimination of alcohol from the body.<sup>3</sup> Therefore, ethyl glucuronide can be a useful diagnostic biomarker for determining recent alcohol use and in monitoring abstinence in alcoholics in alcohol withdrawal treatment programs.<sup>4-7</sup> Ethanol can be produced *in vitro* due to fermentation of glucose in urine samples containing sugars (diabetes), bacteria or yeast when samples are exposed to warm temperatures<sup>8</sup>. In such cases, an ethyl glucuronide test can confirm whether the alcohol in the sample is due to consumption of ethanol or it is formed *in vitro* as a result of fermentation. Currently ethyl glucuronide is monitored by GC/MS and LC-MS/MS.<sup>9-10</sup>

At the present time, there is no consensus cutoff for ethyl glucuronide. Unintentional exposure to ethanol by other means such as hand sanitizers and other products or foods containing ethanol can result in detectable levels of ethyl glucuronide.

The ARK Ethyl Glucuronide II Assay is an *in vitro* diagnostic medical device. The determination of ethyl glucuronide in human urine aids the assessment of compliance for treatment of substance abuse due to excessive consumption of ethanol. Urinary ethyl glucuronide testing has also been used as a tool in the optimal selection of liver transplant candidates and in the early detection of alcohol relapse after liver transplantation.<sup>11</sup>

## 4 Principles of the Procedure

The ARK Ethyl Glucuronide II Assay is a homogeneous enzyme immunoassay technique used for the analysis of ethyl glucuronide 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. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the specimen can be measured in terms of enzyme activity. 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
5077-0001-00	<b>ARK Ethyl Glucuronide II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to ethyl glucuronide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	<b>Reagent [R2] – Enzyme</b> Ethyl glucuronide 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
5077-0001-01	<b>ARK Ethyl Glucuronide II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to ethyl glucuronide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	<b>Reagent [R2] – Enzyme</b> Ethyl glucuronide 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
5077-0001-02	<b>ARK Ethyl Glucuronide II Assay</b>	1 X 500 mL

	<b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to ethyl glucuronide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	
	<b>Reagent [R2] – Enzyme</b> Ethyl glucuronide derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 250 mL

REF	Product Description	Quantity/Volume
5077-0001-03	<b>ARK Ethyl Glucuronide II Assay</b> <b>Reagent [R1] – Antibody/Substrate</b> Rabbit monoclonal antibodies to ethyl glucuronide, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 58 mL
	<b>Reagent [R2] – Enzyme</b> Ethyl glucuronide derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 29 mL

### Reagent Handling and Storage

ARK Ethyl Glucuronide II Assay reagents are provided as 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 Ethyl Glucuronide II 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. Laboratory professional use only.
- 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 for up to 2 months prior to analysis.<sup>12-13</sup>
- 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 the sample 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.<sup>14</sup>
- 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 Ethyl Glucuronide II Assay – **REF** 5077-0001-00, 5077-0001-01, 5077-0001-02, 5077-0001-03

### Materials Required – Provided Separately

ARK Ethyl Glucuronide Calibrator – **REF** 5036-0002-00

ARK Ethyl Glucuronide Calibrator A (Negative) – **REF** 5036-0002-01

ARK Ethyl Glucuronide Calibrator C (500 ng/mL Cutoff) – **REF** 5036-0002-02

ARK Ethyl Glucuronide Calibrator D (1000 ng/mL Cutoff) – **REF** 5036-0002-03

Quality Controls – ARK Ethyl Glucuronide Control (375 ng/mL and 625 ng/mL) – **REF** 5036-0003-00 or ARK Ethyl Glucuronide Control (750 ng/mL and 1250 ng/mL) – **REF** 5036-0003-01

### 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**.

Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Ethyl Glucuronide II 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**

The 500 ng/mL Calibrator C or the 1000 ng/mL Calibrator D can be used as Cutoff Calibrators to distinguish negative and positive samples depending on laboratory specific criteria. Quality Controls are available for each cutoff level. Run the Low (375 ng/mL) and High (625 ng/mL) Controls with Cutoff Calibrator C, and run the Low (750 ng/mL) and High (1250 ng/mL) Controls with Cutoff Calibrator D as Negative and Positive respectively. All qualitative testing results are expressed as enzymatic rate (mA/min). Report test results less than the rate for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate for the Cutoff Calibrator as Positive.

## **Semi-quantitative Results**

To estimate the concentration of ethyl glucuronide, perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with ARK Low and High quality controls according to the established laboratory quality assurance plan. Specimens having concentrations of ethyl glucuronide exceeding 2000 ng/mL may be diluted in ARK Calibrator A (Negative urine).

## **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 28 days based on supporting data.

## **Quality Control (QC)**

Laboratories should establish QC procedures for the ARK Ethyl Glucuronide 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. The ARK Ethyl Glucuronide Control is intended for quality control of the ARK Ethyl Glucuronide II Assay when run in either the qualitative or semi-quantitative mode.

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the respective 500 ng/mL and 1000 ng/mL Cutoff Calibrators used.

## **9 Results and Expected Values**

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

### **Qualitative Analysis – Negative Results**

A specimen that gives a rate value less than the Cutoff Calibrator C or Cutoff Calibrator D rate value as applicable is interpreted as negative; either the specimen does not contain ethyl glucuronide or ethyl glucuronide is present in a concentration below the applicable cutoff level used for this assay.

### **Qualitative Analysis – Positive Results**

A specimen that gives a rate value equal to or greater than the Cutoff Calibrator C or Cutoff Calibrator D rate value as applicable is interpreted as positive, indicating that ethyl glucuronide is present.

### **Semi-quantitative Analysis**

The semi-quantitation of positive levels of ethyl glucuronide enables the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semi-quantitation also permits the laboratory to establish quality control procedures and assess reproducibility. Specimens having concentrations of ethyl glucuronide exceeding 2000 ng/mL may be diluted in ARK Calibrator A (Negative urine).

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 Ethyl Glucuronide II Assay reagents and ARK Ethyl Glucuronide calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Ethyl Glucuronide II Assay indicates only the presence of ethyl glucuronide 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 investigated in the specificity study may interfere with the test and cause false results.
- Exposure to ethanol by other means such as hand sanitizers may cause a false positive result.

## **11 Specific Performance Characteristics**

The data appearing in this section were collected on the Beckman Coulter AU680<sup>®</sup> clinical chemistry analyzer using the ARK Ethyl Glucuronide II Assay.

### **Precision**

Precision was determined by assaying ethyl glucuronide in human urine. Drug-free, negative human urine was supplemented with ethyl glucuronide (0.0 to 2000.0 ng/mL), and both qualitative and semi-quantitative protocols were performed for 20 days, 2 runs per day in quadruplicate (N=160). Both Calibrator C (500 ng/mL) and Calibrator D (1000 ng/mL) were used as cutoffs respectively for assessment of precision in qualitative mode.

#### *Qualitative Precision (500 ng/mL Cutoff)*

<b>Ethyl Glucuronide (ng/mL)</b>	<b>Relative % Cutoff</b>	<b>Result</b>
0	-100	160 Negative
125	-75	160 Negative

250	-50	160 Negative
375	-25	160 Negative
500	0	144 Negative; 16 Positive
625	+25	160 Positive
750	+50	160 Positive
875	+75	160 Positive
1000	+100	160 Positive

*Qualitative Precision (1000 ng/mL Cutoff)*

Ethyl Glucuronide (ng/mL)	Relative % Cutoff	Result
0	-100	160 Negative
250	-75	160 Negative
500	-50	160 Negative
750	-25	160 Negative
1000	0	102 Negative; 58 Positive
1250	+25	160 Positive
1500	+50	160 Positive
1750	+75	160 Positive
2000	+100	160 Positive

*Semi-quantitative Precision (500 ng/mL Cutoff)*

Ethyl Glucuronide (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0	-100	160	18	160 Negative
125	-75	160	136	160 Negative
250	-50	160	266	160 Negative
375	-25	160	388	160 Negative
500	Cutoff	160	518	138 Negative; 22 Positive
625	+25	160	648	160 Positive
750	+50	160	768	160 Positive
875	+75	160	894	160 Positive
1000	+100	160	1018	160 Positive

*Semi-quantitative Precision (1000 ng/mL Cutoff)*

Ethyl Glucuronide (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0	-100	160	18	160 Negative
250	-75	160	266	160 Negative
500	-50	160	518	160 Negative
750	-25	160	768	160 Negative
1000	Cutoff	160	1018	111 Negative; 49 Positive
1250	+25	160	1280	160 Positive
1500	+50	160	1546	160 Positive

Ethyl Glucuronide (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
1750	+75	160	1765	160 Positive
2000	+100	160	2037	160 Positive

### Analytical Recovery

Analytical recovery for the ARK Ethyl Glucuronide II Assay was assessed using the semi-quantitative mode. Drug-free, negative human urine was supplemented with ethyl glucuronide (0.0 to 2000.0 ng/mL). Mean drug concentration observed for six (6) replicates and percentage recovery were calculated.

Concentration Tested (ng/mL)	Observed Value (ng/mL)	Recovery (%)
0	0	NA
50	52	104
100	97	97
250	259	104
500	500	100
750	739	99
1000	976	98
1250	1179	94
1500	1430	95
1750	1670	95
2000	1905	95

### Analytical Specificity

All compounds tested were added to drug-free, negative human urine and tested with the ARK Ethyl Glucuronide II Assay in both qualitative and semi-quantitative modes.

The cross-reactivity of alcohol and glucuronide compounds was evaluated by spiking these compounds into drug-free, negative human urine and evaluated by dose-response to determine the approximate equivalence to the 500 ng/mL and 1000 ng/mL cutoffs. These concentrations were used to determine the percent cross-reactivity according to the formula:

$\% \text{ Cross-reactivity} = (\text{Cutoff concentration} / \text{Concentration approximately equivalent to the 500 ng/mL cutoff or 1000 ng/mL cutoff}) \times 100$

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

*Concentrations (ng/mL) of alcohol glucuronide compounds that produce a result approximately equivalent to the 500 ng/mL and 1000 ng/mL cutoff*

Compound	500 ng/mL Cutoff	1000 ng/mL Cutoff
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	<b>Concentration Approximately Equivalent to the Cutoff (ng/mL)</b>	<b>Cross-reactivity (%)</b>	<b>Concentration Approximately Equivalent to the Cutoff (ng/mL)</b>	<b>Cross-reactivity (%)</b>
Methyl Glucuronide	6199	8.1	14175	3.5
Propyl $\beta$ -D-glucuronide	25996	1.9	58748	0.9

*The following alcohol and glucuronide compounds were negative at the concentrations tested*

<b>Compound</b>	<b>Concentration Tested (ng/mL)</b>	<b>500 ng/mL Cutoff</b>	<b>1000 ng/mL Cutoff</b>
7-hydroxycoumarin Glucuronide	100,000	Negative	Negative
Acetaldehyde	1,000,000	Negative	Negative
Buprenorphine Glucuronide	50,000	Negative	Negative
Butanol	1,000,000	Negative	Negative
D-Glucose	100,000	Negative	Negative
Ethanol	1,000,000	Negative	Negative
Ethyl Sulfate	100,000	Negative	Negative
Ethylene Glycol	1,000,000	Negative	Negative
Glucuronic Acid	100,000	Negative	Negative
Isopropanol	1,000,000	Negative	Negative
L-Glucose	100,000	Negative	Negative
Lorazepam Glucuronide	100,000	Negative	Negative
Methanol	1,000,000	Negative	Negative
Morphine-3-Glucuronide	100,000	Negative	Negative
Morphine-6-Glucuronide	100,000	Negative	Negative
Norbuprenorphine Glucuronide	50,000	Negative	Negative
n-Propanol	1,000,000	Negative	Negative
Oxazepam Glucuronide	50,000	Negative	Negative
p-Nitrophenyl Glucuronide	100,000	Negative	Negative
Temazepam Glucuronide	50,000	Negative	Negative
Trichloroethyl Glucuronide	100,000	Negative	Negative

The following structurally unrelated compounds were negative at the concentrations tested.

<b>Compound</b>	<b>Concentration Tested (ng/mL)</b>	<b>500 ng/mL Cutoff</b>	<b>1000 ng/mL Cutoff</b>
(+)-MDA	100,000	Negative	Negative

11-hydroxy-delta-9-THC	100,000	Negative	Negative
11-nor-9 carboxy THC	100,000	Negative	Negative
1R,2S(-)-Ephedrine	100,000	Negative	Negative
1S,2R(+)-Ephedrine	100,000	Negative	Negative
4-Bromo-2,5-Dimethoxyphenethylamine	100,000	Negative	Negative
6-Acetyl Morphine	100,000	Negative	Negative
6-Acetylcodeine	100,000	Negative	Negative
7-Aminoclonazepam	100,000	Negative	Negative
7-Aminoflunitrazepam	100,000	Negative	Negative
7-Aminonitrazepam	100,000	Negative	Negative
Acetaminophen	500,000	Negative	Negative
Acetylsalicylic acid	1,000,000	Negative	Negative
Alprazolam	100,000	Negative	Negative
Amitriptyline	100,000	Negative	Negative
Amobarbital	100,000	Negative	Negative
Amoxicillin	100,000	Negative	Negative
Amphetamine	100,000	Negative	Negative
Atorvastatin	100,000	Negative	Negative
Benzoyllecgonine	100,000	Negative	Negative
Benzylpiperazine	100,000	Negative	Negative
Bromazepam	100,000	Negative	Negative
Buprenorphine	100,000	Negative	Negative
Bupropion	100,000	Negative	Negative
Butabarbital	100,000	Negative	Negative
Butalbital	100,000	Negative	Negative
Caffeine	100,000	Negative	Negative
Canagliflozin	100,000	Negative	Negative
Cannabidiol	100,000	Negative	Negative
Cannabinol	100,000	Negative	Negative
Carbamazepine	100,000	Negative	Negative
Carisoprodol	100,000	Negative	Negative
Chlordiazepoxide	100,000	Negative	Negative
Chlorpromazine	100,000	Negative	Negative
Cimetidine	100,000	Negative	Negative
Ciprofloxacin	100,000	Negative	Negative
cis-Tramadol	100,000	Negative	Negative
Citalopram	100,000	Negative	Negative
Clobazam	100,000	Negative	Negative
Clomipramine	100,000	Negative	Negative
Clonazepam	100,000	Negative	Negative
Clopidogrel	100,000	Negative	Negative
Cocaine	100,000	Negative	Negative
Codeine	100,000	Negative	Negative
Cotinine	100,000	Negative	Negative

Cyclobenzaprine	100,000	Negative	Negative
Delta-9-THC	100,000	Negative	Negative
Demoxepam	100,000	Negative	Negative
Desalkylflurazepam	100,000	Negative	Negative
Desmethyl Ofloxacin	100,000	Negative	Negative
Desipramine	100,000	Negative	Negative
Dextromethorphan	100,000	Negative	Negative
Diazepam	100,000	Negative	Negative
Dihydrocodeine	100,000	Negative	Negative
Diphenhydramine	100,000	Negative	Negative
Doxepin	100,000	Negative	Negative
Ecgonine	100,000	Negative	Negative
Ecgonine methyl ester	100,000	Negative	Negative
EDDP	100,000	Negative	Negative
Ephedrine	100,000	Negative	Negative
Ethylmorphine	100,000	Negative	Negative
Fenfluramine	100,000	Negative	Negative
Fenofibrate	100,000	Negative	Negative
Fentanyl	100,000	Negative	Negative
Flunitrazepam	100,000	Negative	Negative
Fluoxetine	100,000	Negative	Negative
Fluphenazine	100,000	Negative	Negative
Flurazepam	100,000	Negative	Negative
Heroin	100,000	Negative	Negative
Hexobarbital	100,000	Negative	Negative
Hydrocodone	100,000	Negative	Negative
Hydromorphone	100,000	Negative	Negative
Ibuprofen	500,000	Negative	Negative
Imipramine	100,000	Negative	Negative
Ketamine	100,000	Negative	Negative
Lamotrigine	100,000	Negative	Negative
Levorphanol	100,000	Negative	Negative
Lidocaine	100,000	Negative	Negative
Lorazepam	100,000	Negative	Negative
Lorazepam Glucuronide	100,000	Negative	Negative
Lormetazepam	100,000	Negative	Negative
LSD	100,000	Negative	Negative
Maprotiline	100,000	Negative	Negative
MDEA	100,000	Negative	Negative
MDMA	100,000	Negative	Negative
Meperidine	100,000	Negative	Negative
Meprobamate	100,000	Negative	Negative
Metformin	100,000	Negative	Negative
Methadone	100,000	Negative	Negative

Methaqualone	100,000	Negative	Negative
Methylphenidate	100,000	Negative	Negative
Metronidazole	300,000	Negative	Negative
Midazolam	100,000	Negative	Negative
Morphine	100,000	Negative	Negative
Nalbuphine	100,000	Negative	Negative
Nalorphine	100,000	Negative	Negative
Naloxone	100,000	Negative	Negative
Naltrexone	100,000	Negative	Negative
Naproxen	100,000	Negative	Negative
N-desmethyltapentadol	100,000	Negative	Negative
Nitrazepam	100,000	Negative	Negative
Norbuprenorphine	100,000	Negative	Negative
Norcodeine	100,000	Negative	Negative
Nordiazepam	100,000	Negative	Negative
Normorphine	100,000	Negative	Negative
Norpropoxyphene	100,000	Negative	Negative
Norpseudoephedrine	50,000	Negative	Negative
Nortriptyline	100,000	Negative	Negative
Ofloxacin	100,000	Negative	Negative
Omeprazole	100,000	Negative	Negative
Ondansetron	100,000	Negative	Negative
Oxazepam	100,000	Negative	Negative
Oxycodone	100,000	Negative	Negative
Oxymorphone	100,000	Negative	Negative
Phencyclidine	100,000	Negative	Negative
Phenobarbital	100,000	Negative	Negative
Phentermine	100,000	Negative	Negative
Phenylpropanolamine	100,000	Negative	Negative
Phenytoin	100,000	Negative	Negative
PMA	100,000	Negative	Negative
Prazepam	100,000	Negative	Negative
Propranolol	100,000	Negative	Negative
Propoxyphene	100,000	Negative	Negative
Protriptyline	100,000	Negative	Negative
R,R(-)-Pseudoephedrine	100,000	Negative	Negative
Ranitidine	100,000	Negative	Negative
Ritalinic Acid	100,000	Negative	Negative
S(+)-Methamphetamine	100,000	Negative	Negative
S,S(+)-Pseudoephedrine	100,000	Negative	Negative
Salicylic Acid	100,000	Negative	Negative
Secobarbital	100,000	Negative	Negative
Sertraline	100,000	Negative	Negative
Sufentanil Citrate	50,000	Negative	Negative

Talwin	100,000	Negative	Negative
Temazepam	100,000	Negative	Negative
Thebaine	100,000	Negative	Negative
Theophylline	100,000	Negative	Negative
Thioridazine	100,000	Negative	Negative
Tramadol	100,000	Negative	Negative
Trazodone	100,000	Negative	Negative
Triazolam	100,000	Negative	Negative
Trifluoromethylphenyl-piperazine	100,000	Negative	Negative
Trimipramine	100,000	Negative	Negative
Venlafaxine	1,000,000	Negative	Negative
Xylazine	100,000	Negative	Negative
Zolpidem	100,000	Negative	Negative
Coca ethylene	100,000	Negative	Negative

### Interference – Endogenous Substances

The potential interference of endogenous substances on recovery of ethyl glucuronide using the ARK Ethyl Glucuronide II Assay was assessed by adding known amounts of potentially interfering substances into the  $\pm 25\%$  controls for both the cutoffs, 500 ng/mL and 1000 ng/mL and testing the samples for recovery of ethyl glucuronide. No interference was observed by the addition of the compounds up to the concentrations listed below, except for boric acid, which showed interference at the 1000 ng/mL cutoff.

*The following endogenous compounds at 500 ng/mL cutoff*

Compounds	Concentration (mg/dL)	375 ng/mL Ethyl Glucuronide (POS/NEG)	625 ng/mL Ethyl Glucuronide (POS/NEG)
Acetone	1000	NEG	POS
Ascorbic Acid	560	NEG	POS
Bilirubin	2	NEG	POS
Boric Acid	1% w/v	NEG	POS
Creatinine	500	NEG	POS
Galactose	10	NEG	POS
Glucose	3000	NEG	POS
Hemoglobin	500	NEG	POS
Human Albumin	500	NEG	POS
Oxalic Acid	100	NEG	POS
Human $\gamma$ Globulin	500	NEG	POS
Riboflavin	7.5	NEG	POS
NaCl	4000	NEG	POS
Urea	2000	NEG	POS

*The following endogenous compounds at 1000 ng/mL cutoff*

Compounds	Concentration (mg/dL)	750 ng/mL Ethyl Glucuronide (POS/NEG)	1250 ng/mL Ethyl Glucuronide (POS/NEG)
Acetone	1000	NEG	POS
Ascorbic Acid	560	NEG	POS
Bilirubin	2	NEG	POS
Creatinine	500	NEG	POS
Galactose	10	NEG	POS
Glucose	3000	NEG	POS
Hemoglobin	500	NEG	POS
Human Albumin	500	NEG	POS
Oxalic Acid	100	NEG	POS
Human $\gamma$ Globulin	500	NEG	POS
Riboflavin	7.5	NEG	POS
NaCl	4000	NEG	POS
Urea	2000	NEG	POS

#### Interference – Boric Acid

One percent (1%) w/v of boric acid was added into ethyl glucuronide-spiked urine ( $\pm$  25% of the 1000 ng/mL cutoff concentration). Results are provided in the table below.

Compounds	Concentration Tested	750 ng/mL Ethyl Glucuronide (POS/NEG)	1250 ng/mL Ethyl Glucuronide (POS/NEG)
Boric Acid	1% w/v	NEG	NEG

#### Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.0045 g/mL to 1.0242 g/mL and pH values ranging from 3.0 to 11.0 were tested with ethyl glucuronide at concentrations  $\pm$ 25% of the 500 ng/mL and 1000 ng/mL cutoffs. No interference was observed.

#### Comparative Analysis

A total of one hundred and ninety nine (199) clinical urine specimens were analyzed by ARK Ethyl Glucuronide II Assay. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory and used an ethyl glucuronide cutoff of 50.0 ng/mL. The ARK Ethyl Glucuronide II Assay (500 ng/mL and 1000 ng/mL cutoffs) distinguished positive and negative results: 100% clinical sensitivity and 100% clinical specificity at the 500 ng/mL cutoff, and 96% clinical sensitivity and 100% clinical specificity at the 1000 ng/mL cutoff.

<b>LC-MS/MS</b>
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		(+)	(-)
<b>ARK Ethyl Glucuronide II Assay (500 ng/mL Cutoff)</b>	(+)	100	0
	(-)	0	99

<b>LC-MS/MS</b>			
		(+)	(-)
<b>ARK Ethyl Glucuronide II Assay (1000 ng/mL Cutoff)</b>	(+)	96	0
	(-)	4*	99

*\*Discordant Result Summary*

Sample ID	ARK Qualitative (Negative/Positive)	ARK Semi-quantitative (ng/mL)	LC-MS/MS Ethyl Glucuronide (ng/mL)
044	Negative	942.0	900.0
045	Negative	782.9	580.0
050	Negative	714.5	600.0
072	Negative	661.6	720.0

\*Samples contained concentrations of ethyl glucuronide between the cutoffs, 500 ng/mL and 1000 ng/mL, for the ARK assay as confirmed by LC-MS/MS.

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## 13 Trademarks

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ARK Diagnostics, Inc.  
Fremont, CA 94538 USA

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