

Catalog No.: 10011723 (20 mL Kit)
10011297 (70 mL Kit)
10011226 (500 mL Kit)

Intended Use

The DRI® Ethyl Glucuronide Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of Ethyl Glucuronide in human urine at cutoffs of 500 and 1000 ng/mL.

This assay provides only a preliminary analytical test result. A more specific alternative method must be used in order to obtain a confirmed analytical result. Gas Chromatography/Liquid chromatography mass spectrometry (GC/MS) and Liquid chromatography/tandem mass spectrometry (LC/MS/MS) are the preferred confirmatory methods. 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

Ethyl Glucuronide (EtG) is a direct metabolite of ethanol, which is formed by enzymatic conjugation of ethanol with glucuronic acid.^{1,2} Alcohol in urine is normally detected for only a few hours, whereas EtG can be detected up to several days even after complete elimination of alcohol from the body.³ Therefore, EtG can be a useful diagnostic biomarker for determining recent alcohol use and in monitoring abstinence in alcoholics in alcohol withdrawal treatment programs.⁴⁻⁷ Ethanol can be produced *in vitro* due to fermentation of urine samples containing sugars (diabetes), bacteria or yeast when samples are exposed to warm temperatures.⁸ In such cases, EtG test can be used, as a confirmatory test to determine if the alcohol in the sample is due to consumption of alcohol or it is formed *in vitro* as a result of fermentation. Currently EtG is monitored by GC/MS and LC/MS/MS.⁹⁻¹⁰

The DRI® Ethyl Glucuronide Assay is supplied as a liquid ready-to-use homogeneous enzyme immunoassay. The assay uses specific antibodies that can detect Ethyl Glucuronide without any significant cross-reactivity to other glucuronide 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. Active enzyme converts NAD to NADH resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

Reagents

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

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

Additional Materials Required (sold separately):

1664 DRI® DAU Negative Calibrator, 10 mL
10011208, DRI® Ethyl Glucuronide Calibrator 100, 10 mL
10011210, DRI® Ethyl Glucuronide Calibrator 500, 10 mL
10011212, DRI® Ethyl Glucuronide Calibrator 1000, 10 mL
10011213, DRI® Ethyl Glucuronide Calibrator 2000, 10 mL
10011209, DRI® Ethyl Glucuronide Control Kit 500, 2 x 25 mL
10011211, DRI® Ethyl Glucuronide Control Kit 1000, 2 x 25 mL

Precautions and Warnings

1. This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. Reagents used in the assay components contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metalazides. When disposing of such reagents, always flush with a large volume of water to prevent azide build up.
3. Do not use reagents beyond their expiration dates

Reagent Preparation and Storage

The reagents are ready-to-use; no additional preparation is required. Reagents should be stored refrigerated, 2° to 8°C. 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. Fresh urine specimens are suggested.

The *Mandatory Guidelines for Federal Workplace Drug Testing Programs* recommends that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units. Urine samples must be stored refrigerated at all times.

Samples within a pH range of 4.5 to 11 are suitable for testing with this assay.

An effort should be made to keep pipetted samples free of gross debris. Centrifuge highly turbid specimens before analysis. Adulteration of the urine samples 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 range, as determined by laboratory procedures and guidelines. If results fall outside of the established ranges, assay results are invalid. For qualitative analysis, use either 500 ng/mL or 1000 ng/mL calibrator as cutoff level. For semi-quantitative analysis, use all calibrators. All QC requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

Qualitative

Either the 500 ng/mL or 1000 ng/mL calibrators can be used as a Cutoff reference for distinguishing "positive" from "negative" samples. A sample that exhibits a change in absorbance value (ΔA) equal to or greater than that obtained with cutoff calibrator is considered positive. A sample that exhibits a change in absorbance value (ΔA) lower than that obtained with cutoff calibrator is considered negative.

Semi-quantitative

A rough estimate of Ethyl Glucuronide concentration in the samples can be obtained by running a standard curve with all calibrators and quantitating samples off the standard curve. When the concentration of EtG in the sample is greater than the highest calibrator, it may be diluted with negative calibrator and retested.

Reportable Range

The DRI® Ethyl Glucuronide Assay is designed for semi-quantitative use in the range between 100 ng/mL, the lowest calibrator and 2000 ng/mL, the value of the high calibrator.

Limitations

1. A positive result using the DRI® Ethyl Glucuronide Assay indicates only the presence of Ethyl Glucuronide and does not necessarily correlate with the extent of physiological and psychological effects.
2. Performance characteristics for the DRI® Ethyl Glucuronide Assay have not been established with body fluids other than human urine.
3. This DRI® Ethyl Glucuronide Assay was validated on analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local Thermo Fisher Scientific representative.
4. Care should be taken when reporting results since there are many factors, e.g., fluid intake and other biologic factors, that may influence urine test result.
5. It is possible that substances other than those investigated in the specificity study may interfere with the test and cause false results.

Typical Performance Characteristics

Typical performance results obtained on the Hitachi 917 analyzer are shown below. The results obtained in your laboratory may differ from these data. For additional analyzer specific performance data, refer to the analyzer specific application sheet.

Precision

The DRI® Ethyl Glucuronide controls (375, 625, 750 and 1250 ng/mL) and cutoff calibrators (500 and 1000 ng/mL) were tested in qualitative (mA/min) and semi-quantitative (ng/mL) mode using a modified NCCLS protocol. Results presented below were generated by testing all samples in replicates of 6, twice per day for 10 days.

Qualitative (mA/min)

Calibrator/Control	500 ng/mL cutoff					
	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
N=120						
375	392	2.1	0.5	392	2.9	0.7
500	417	2.1	0.5	417	3.1	0.7
625	439	2.0	0.5	439	2.7	0.6

Qualitative (mA/min)

Calibrator/Control	1000 ng/mL cutoff					
	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
N=120						
750	461	2.4	0.5	461	3.4	0.7
1000	494	2.7	0.6	494	3.4	0.7
1250	524	2.7	0.5	524	3.8	0.7

Semi-quantitative (ng/mL)

Calibrator/Control	500 ng/mL cutoff					
	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
N=120						
375	373	11.3	3.0	373	18.1	4.9
500	502	10.5	2.1	502	19.4	3.9
625	623	13.2	2.1	623	22.3	3.6

Semi-quantitative (ng/mL)

Calibrator/Control	1000 ng/mL cutoff					
	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
N=120						
750	756	16.9	2.2	756	31.2	4.1
1000	993	21.1	2.1	993	34.3	3.5
1250	1232	23.0	1.9	1232	43.5	3.5

Interference with Endogenous Substances

The potential interference of pH and endogenous physiologic substances on recovery of Ethyl Glucuronide using the DRI® Ethyl Glucuronide 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.

Interfering Substance	Final Concentration mg/dL
Actaminophen	10
Acetone	1000
Acetyl Salicylic Acid	10
Ascorbic Acid	190
Caffeine	10
Creatinine	400
Ethanol	10
Galactose	10
Glucose	3000
Hemoglobin	300
Human Serum Albumin	500
Ibuprofen	10
Oxalic Acid	30
Riboflavin	3.75
Sodium Chloride	900
Urea	1000
pH	4.5-11.0

Cutoff Characterization-Spike Recovery

Cutoff calibrators, 500 ng/mL and 1000 ng/mL and $\pm 25\%$ controls were prepared by spiking Ethyl Glucuronide into EtG free negative urine. The cutoff calibrators and controls were tested (n=21) in both the qualitative and semi-quantitative modes. The qualitative data were analyzed for precision and detection accuracy of controls and semi-quantitative data were analyzed for % recovery and precision. The results indicated that all four controls recovered accurately in qualitative mode, negative controls as negative (rate below the C/O calibrator rate) and positive controls as positive (rate above the C/O calibrator rate). In semi-quantitative mode all controls were recovered within $\pm 10\%$ from nominal values. The precision was $<1.0\%$ CV in qualitative mode $<5.0\%$ CV in semi-quantitative mode.

Specificity

The cross-reactivity of parent compound ethanol and glucuronide compounds that are commonly found in urine was tested in the assay using 500 ng/mL cutoff calibrator. The cross-reactant solutions were prepared by adding known amount of each compound to Ethyl Glucuronide free urine. All the compounds produced a negative result at the concentrations listed in the table below.

Compound	Conc. (ng/mL)
Acetaldehyde	10,000
Alprazolam Glucuronide	10,000
Buprenorphine Glucuronide	10,000
Butanol	10,000
D-Glucose	10,000
Ethanol	100,000
Ethylene Glycol	10,000
Glucuronic Acid	10,000
HydroxyCourmarin Glucuronide	10,000
Isopropanol	10,000
Lorazepam Glucuronide	10,000
Methanol	10,000
Methyl Glucuronide	5,000
Morphine -3- Glucuronide	10,000
Morphine-6-Glucuronide	10,000
Norbuprenorphine Glucuronide	10,000
n-Propanol	10,000
Oxazepam Glucuronide	10,000
p-Nitrophenyl Glucuronide	10,000
Termazepam Glucuronide	10,000
Testosterone Glucuronide	10,000

The cross-reactivity of structurally unrelated compounds was tested in the assay using 500 ng/mL as cutoff calibrator. All the compounds produced a negative result at the concentrations listed in the table below.

Compound	Conc. (µg/mL)
6-Acetyl Morphine	500
Acetaminophen	500
Acetylsalicylic acid	500
Amitriptyline	100
Amoxicillin	100
Amphetamine	1000
Benzoylcegonine	1000
Caffeine	100
Carbamazepine	500
Chlorpromazine	100
Clomipramine	100
Cimetidine	500
Codeine	1000
Desipramine	1000
Dextromethorphan	200
Dihydrocodeine	1000
Doxepine	200
Ephedrine	2000
Fentanyl	200
Fluoxetine	1000
Fluphenazine	500
Heroin	1000
Hydrocodone	200
Hydromorphone	200
Ibuprofen	1000
Imipramine	1000
Levorphanol	500
Maprotiline	1000
Meperidine	1000
Methadone	1000
Metronidazole	500
Morphine	1000
Morphine-3-Glucuronide	1000
Nalbuphine	1000
Naltrexone	3000
Norcodeine	1000
Normorphine	1000
Nortriptyline	500
Oxazepam	500
Phencyclidine	1000
Phenobarbital	1000
Ranitidine	500
Secobarbital	1000
Talwin	500
Thebaine	100
Thioridazine	500
Tramadol	500
Oxycodone	500

Sensitivity

The sensitivity of the assay as evaluated by the EP Evaluator 7.0 was 15.3 ng/mL

Linearity

The assay linearity was determined by testing the dilution recovery of a series of Ethyl Glucuronide samples in the assay. A urine sample containing 2000 ng/mL Ethyl Glucuronide was serially diluted with EtG free urine at 25% increments from cutoff calibrators. These samples were tested in the assay in both the qualitative and semi-quantitative modes. All the samples were recovered within $\pm 20\%$ of expected values in the semi-quantitative mode and expected rate (mA/min) in qualitative mode indicating that the assay is linear up to 2000 ng/mL.

Accuracy

One hundred and eighty four samples were analyzed by DRI® Ethyl Glucuronide Assay in both the qualitative and semi-quantitative modes and the results were compared to LC/MS/MS method. In both the qualitative and semi-quantitative modes, the positive sample agreement between DRI® EtG Assay and LC/MS/MS was 96%. The results obtained by both the qualitative and semi-quantitative modes are summarized below.

Qualitative: Out of 184 samples, using 500 ng/mL cutoff, 94 samples were detected as positive and 85 samples as negative and at 1000 ng/mL cutoff 44 samples were detected as positive and 138 samples were detected as negative by both the immunoassay and LC/MS/MS. The overall concordance between the immunoassay and LC/MS/MS was 97%. There were five discordant samples at 500 ng/mL cutoff and two discordant samples at 1000 ng/mL cutoff.

Semi-Quantitative: In semi-quantitative mode, samples with EtG concentration >500 ng/mL and 1000 ng/mL were considered positive in the immunoassay. Out of 184 samples, 94 samples were detected as positive and 85 samples as negative by both the immunoassay and LC/MS/MS methods.

		DRI® EtG Assay	
		+	-
LC/MS/MS	+	94	3*
	-	2 [†]	85

* Samples were the same samples as in 500 ng/mL qualitative mode.

[†] Samples were the same samples as in 500 ng/mL qualitative mode.

		500 ng/mL C/O DRI® EtG Assay		1000 ng/mL C/O DRI® EtG Assay	
		+	-	+	-
LC/MS/MS	+	94	3*	44	2 [†]
	-	2 [†]	85	0	138

* Two of the three samples were borderline negative by the immunoassay.

One sample was borderline positive by LC/MS/MS.

[†] Samples were borderline positive in the immunoassay.

[‡] Samples were borderline negative in the immunoassay. LC/MS/MS values were between 1000 and 1250 ng/mL.

References

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