DRI® Ethyl Glucuronide Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

REF 10015626 (3 x 18 mL Kit)
10011723 (18 mL Kit)
10011297 (68 mL Kit)

CAUTION: FOR EXPORT USE ONLY.
NOT FOR SALE IN THE UNITED STATES.

Intended Use

The Thermo Scientific™ DRI Ethyl Glucuronide Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of Ethyl Glucuronide in human urine at cutoffs of 500 ng/mL 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] Currently EtG is monitored by GC/MS and LC/MS/MS.[4,5]

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):

REF	Kit Description
10011207	DRI Ethyl Glucuronide Negative Calibrator, 25 mL
10011208	DRI Ethyl Glucuronide Calibrator 100 ng/mL, 10 mL
10011210	DRI Ethyl Glucuronide Calibrator 500 ng/mL, 10 mL
10011212	DRI Ethyl Glucuronide Calibrator 1000 ng/mL, 10 mL
10011213	DRI Ethyl Glucuronide Calibrator 2000 ng/mL, 10 mL
10012135	DRI Ethyl Glucuronide Control 375 ng/mL, 25 mL
10012136	DRI Ethyl Glucuronide Control 625 ng/mL, 25 mL
10012137	DRI Ethyl Glucuronide Control 750 ng/mL, 25 mL
10012138	DRI Ethyl Glucuronide Control 1250 ng/mL, 25 mL

⚠ Precautions and Warnings

DANGER: DRI Ethyl Glucuronide (EtG) Assay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% drug-specific antibody.
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

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

1. This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. Reagents used in the assay components contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.
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 recommend 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 when not in use.

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 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 the 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 the 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. Performance characteristics for the DRI Ethyl Glucuronide Assay have not been established with body fluids other than human urine.
2. 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.
3. Care should be taken when reporting results since there are many factors, e.g., fluid intake and other biologic factors, that may influence a urine test result.
4. 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

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		
N=120	Mean	SD	% CV	Mean	SD	% CV
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		
N=120	Mean	SD	% CV	Mean	SD	% CV
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		
N=120	Mean	SD	% CV	Mean	SD	% CV
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		
N=120	Mean	SD	% CV	Mean	SD	% CV
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

Cutoff Characterization-Spike Recovery

Cutoff calibrators, 500 ng/mL and 1000 ng/mL and ±25% controls were prepared by spiking Ethyl Glucuronide into EtG free negative urine. The cutoff calibrator 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 controls were recovered within ±10% from nominal values. The precision was <1.0% CV in qualitative mode <5.0% CV in semi-quantitative mode.

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 ±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

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
Amitryptyline	100
Amoxicillin	100
Amphetamine	1000
Benzoylecgonine	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
Fluoxethine	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
Nortryptyline	500
Oxazepam	500
Oxycodone	500
Phencyclidine	1000
Phenobarbital	1000
Ranitidine	500
Secobarbital	1000
Talwin	500
Thebaine	100
Thioridazine	500
Tramadol	500

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 ±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 the 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.

500 ng/mL C/O LC – MS/MS

DRI EtG Assay	LC – MS/MS +	LC – MS/MS -
+	94	2¥
-	3*	85

1000 ng/mL C/O LC – MS/MS

DRI EtG Assay	LC – MS/MS +	LC – MS/MS -
+	44	0
-	2♠	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.

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 +	LC – MS/MS -
+	94	2*
-	3¥	85

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

References

1. Ethyl Glucuronide: An unusual Ethanol Metabolite in Humans.Synthesis, Analytical Data, and Determination in Serum and Urine. Schmitt G., et al. Journal of Analytical Toxicology. 1995, 19:91-94.
2. Comparison of Urinary Excretion Characteristics of Ethanol and Ethyl Glucuronide. Dahl H., et al. Journal of Analytical Toxicology. 2002, 26:201-204.
3. Ethyl Glucuronide- the direct ethanol metabolite on the threshold from science to routine use. Wurst FM et al. Addiction. 2003, 98 (S2) 51-61.
4. Preliminary immunochemical test for the determination of Ethyl Glucuronide in serum and urine: Comparison of screening method results with Gas Chromatography- Mass spectrometry. Zimmer H., et al. Journal of Analytical Toxicology. 2002, 26:11-16.
5. Confirmatory Analysis of Ethyl Glucuronide in urine by liquid chromatography/ Elctrospray Ionization/Tandem Mass Spectrometry according to forensic guidelines. Weinmann W. et al. J. Am. Soc. Mass Spectrom. 2004, 15(2):188-193.

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