DRI® Benzodiazepine Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

REF 10015644 (3 x 18 mL)
0039 (100 mL Kit)
0040 (500 mL Kit)

Intended Use
The DRI Benzodiazepine enzyme immunoassay is a homogeneous assay intended for the qualitative and semiquantitative determination of benzodiazepines in human urine.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1,2] 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
Benzodiazepines are sedative-hypnotic drugs, which are subject to abuse. Benzodiazepines are structurally similar and include a wide variety of drugs such as alprazolam, chlordiazepoxide, diazepam, lorazepam, oxazepam and triazolam. They are absorbed and metabolized at different rates, resulting in various psychoactive effects. Therefore, the detection of benzodiazepines or their metabolites in urine can be used as an indicator of benzodiazepine abuse.

The DRI® Benzodiazepine Assay is a homogeneous enzyme immunoassay[3] with liquid ready-to-use reagents. The assay uses a specific antibody which can detect most benzodiazepines and their metabolites in urine. The assay is based on the competition of an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled drug and the drug from the urine sample for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the enzyme-labeled drug is bound by the specific antibody and the enzyme activity is inhibited. This phenomenon creates a relationship between drug concentration in urine and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents
Antibody/Substrate Reagent.
Contains sheep polyclonal anti-benzodiazepine antibodies, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent.
Contains benzodiazepine derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Material Required (sold separately):

REF	Kit Description
1664	DRI Negative Calibrator, 10 mL
1388	DRI Negative Calibrator, 25 mL
1588	DRI Multi-Drug Urine Calibrator 1, 10 mL
1589	DRI Multi-Drug Urine Calibrator 1, 25 mL
1591	DRI Multi-Drug Urine Calibrator 2, 10 mL
1592	DRI Multi-Drug Urine Calibrator 2, 25 mL
1594	DRI Multi-Drug Urine Calibrator 3, 10 mL
1595	DRI Multi-Drug Urine Calibrator 3, 25 mL
1597	DRI Multi-Drug Urine Calibrator 4, 10 mL
1598	DRI Multi-Drug Urine Calibrator 4, 25 mL
DOAT-4	MAS® DOA Total – Level 4
DOAT-5	MAS® DOA Total – Level 5

⚠ Precautions and Warnings
This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.

DANGER: The DRI Benzodiazepine enzyme immunoassay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Sheep).
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 becomes 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.

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.

Do not use the reagents beyond their expiration dates.

Reagent Preparation and Storage
The reagents are ready to use. No reagent preparation is required. All assay components when stored properly at 2-8°C, are stable until the expiration date indicated on the label.

Specimen Collection and Handling
Collect urine specimens in plastic or glass containers. Testing of fresh urine specimens is suggested.

The Mandatory Guidelines for Federal Workplace Drug Testing Programs; Final Guidelines recommends that specimens that do not receive an initial test within 7 days of arrival in the laboratory should be placed into secure refrigeration units.

Samples within a pH range of 3 to 11 are suitable for testing with this assay.

An effort should be made to keep pipetted samples free of gross debris. It is recommended that highly turbid specimens be centrifuged before analysis. Adulteration of the urine sample 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
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 assay.

Refer to the specific application instructions of each analyzer for chemistry parameters before performing the assay.

Quality Control and Calibration
Qualitative analysis
For qualitative analysis of samples, use the 200 ng/mL calibrator as a cutoff level. The DRI Multi-Drug Urine Calibrator 2, which contains 200 ng/mL oxazepam, is used as a cutoff reference for distinguishing "positive" from "negative" samples.

Semiquantitative analysis
For semiquantitative analysis, use all calibrators.

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. Control results must fall within established ranges as determined by your laboratory. If results fall outside of established ranges, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values
Qualitative results
A sample that exhibits a change in absorbance (ΔA) value equal to or greater than the value obtained with the cutoff calibrator is considered positive. A sample that exhibits a change in absorbance (ΔA) value lower than the value obtained with the cutoff calibrator is considered negative.

Semiquantitative results
A rough estimate of drug concentration in the samples can be obtained by running a standard curve with all calibrators and quantitating samples off the standard curve. Refer to the analyzer specific protocol sheets.

Limitations
1. A positive result from this assay indicates only the presence of benzodiazepines and does not necessarily correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by another nonimmunological method such as GC, TLC or GC/MS.
3. The test is designed for use with human urine only.
4. It is possible that other substances and/or factors (eg, technical or procedural) not listed in the specificity table may interfere with the test and cause false results.

Typical Performance Characteristics
Performance results obtained on a Hitachi 717 analyzer are shown below.[4] The results obtained in your laboratory may differ from these data.

Precision
Quality Control samples at 150 and 250 ng/mL and the cutoff calibrator (200 ng/mL) were tested in the qualitative mode using a modified NCCLS protocol. Results presented below were generated by testing all samples in replicates of 6, twice per day for 5 days for a total N=60 replicates.

Qualitative

Calibrator/ Control	Within-run Precision			Total Precision		
	Mean (mA/min)	SD (mA/min)	% CV	Mean (mA/min)	SD (mA/min)	% CV
150 ng/mL	375	3.26	0.87	375	6.69	1.78
200 ng/mL	406	3.24	0.80	406	7.32	1.80
250 ng/mL	434	3.91	0.90	434	8.00	1.84

Sensitivity
The sensitivity of the assay as evaluated by the EP Evaluator 7.0 was 5.8 ng/mL.

Accuracy
One hundred clinical urine samples were tested by a commercially available EIA assay and the DRI Benzodiazepine assay. All positive samples by the commercial EIA were tested by GC/MS. The overall concordance between the two immunoassay methods was 92%. The overall concordance between GC/MS and DRI Benzodiazepine assay was 95%.

		Commercial Immunoassay	
		+	-
DRI Benzodiazepine Assay	+	42	1*
	-	7¥	50

¥ 3 samples were negative by GC/MS; 3 samples contained benzodiazepine compounds below the detection level by DRI Benzodiazepine Assay.
* Borderline positive by DRI Benzodiazepine Assay.

		Commercial Immunoassay	
		+	-
DRI Benzodiazepine Assay	+	42	1‡
	-	4†	53

† 3 samples contained benzodiazepine compounds below the detection level by DRI Benzodiazepine Assay.
‡ Sample quantity not sufficient for further analysis.

Specificity
Various benzodiazepine compounds and their metabolites were tested for cross-reactivity in the assay. The table below listed the concentrations of compounds that produced positive results.

Compound	Concentration (ng/mL)
Alprazolam	85
7-Aminoclonazepam	2500
Bromazepam	170
Chlordiazepoxide	750
Clobazam	110
Clonazepam	150
Clorazepate	85
Delorazepam	110
Desmethyldiazepam	75
Diazepam	70
Flunitrazepam	95
Flurazepam	100
Lorazepam	650
Lormetazepam	225
Medazepam	170
Nitrazepam	100
Norfludiazepam	85
Prazepam	95
Temazepam	115
Triazolam	90

The following potentially interfering compounds tested negative at the concentrations listed below.

Compound	Concentration (µg/mL)
Acetaminophen	1000
Acetylsalicylic acid	1000
Amphetamine	1000
Caffeine	100
Codeine	1000
Dextromethorphan	1000
Diphenhydramine	500
Fluoxetine	500
Methadone	1000
Morphine	200
Nor-Fluoxetine	500
Nor-Sertraline	1000
Oxaprozin	50
Paroxetine	500
Phencyclidine	1000
Propoxyphene	1000
Secobarbital	1000
Sertraline	500

References

1. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Mandatory Guidelines for Federal Workplace Drug Testing Program. National Institute on Drug Abuse. Federal Register Vol. 53, No 69, pp 11970 (1988).
3. Rubenstein KE, Schneider RS, and EF Ullman: Homogeneous enzyme immunoassay: a new immunochemical technique. Biochem Biophys Res Commun 47:846-851 (1972).
4. Data on file at Microgenics, a part of Thermo Fisher Scientific.

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