DRI® Benzodiazepine Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

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

Intended Use

The DRI® Benzodiazepine Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semi-quantitative determination of the presence of benzodiazepines and their metabolites in human urine at a cutoff concentration of 200 ng/mL. The assay is intended to be used in laboratories and provides a rapid analytical screening procedure to detect benzodiazepines in human urine. The assay is designed for use with a number of clinical chemistry analyzers. This assay is calibrated against Oxazepam. This product is intended to be used by trained professionals only.

The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/tandem mass spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) or Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the preferred confirmatory method.[1,2]

Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are used. For *In Vitro Diagnostic* Use Only.

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

REAGENT ***Antibody/Substrate Reagent (A):***
Contains sheep polyclonal anti-benzodiazepine antibodies, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

REAGENT ***Enzyme Conjugate Reagent (E):***
Contains benzodiazepine 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
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

The reagents are harmful if swallowed.

DANGER:

1. The reagents contain ≤ 0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Sheep). Avoid contact with skin and mucous membranes. Avoid inhalation. May cause skin or inhaled allergic reaction.
2. In the case of accidental spill, clean and dispose of material according to your laboratory's Standard Operating Procedure, local, and state regulations.
3. In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).
4. Reagents used in the assay components contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Refer to Safety Data Sheet for additional precautions, handling instructions, and accidental exposure treatment.

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.

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components when stored at 2-8ºC, are stable until the expiration date indicated on the label. Do not use reagents beyond the expiration dates.

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Specimens kept at room temperature that do not receive initial test within 7 days[5] of arrival at the laboratory may be placed into a secure refrigeration unit at 2-8ºC for two months.[6] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20ºC.[6, 7]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[4]

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. An effort should be made to keep pipetted samples free of gross debris. It is recommended that grossly turbid specimens be centrifuged before analysis. Frozen samples should be thawed and mixed prior to 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

The DRI Benzodiazepine Assay is intended for use on automated clinical analyzers capable of maintaining a constant temperature, pipetting, 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.

Qualitative analysis
For qualitative analysis, use the DRI Multi-Drug Urine Calibrator 2, which contains Oxazepam 200 ng/mL, as a cutoff reference for distinguishing "positive" from "negative" samples.

Semi-quantitative analysis
For semi-quantitative analysis, use all five calibrators.

Quality Control and Calibration

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. It is recommended that two controls be run; a positive control and a negative control. Base assessment of quality control on the values obtained for the controls, which should fall within specified limits. If any trends or sudden shifts in values are detected, review all operating parameters. Contact Customer Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements. Each laboratory should establish its own quality control testing frequency.

Results and Expected Values

Qualitative:

The DRI Benzodiazepine cutoff calibrator (200 ng/mL) is used as a reference in distinguishing between "positive" and "negative" samples. 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.

Semi-quantitative:

An estimate of benzodiazepine drug concentrations 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. Concentrations of drug values only can be used for making controls and dilutions for confirmatory testing.

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 non-immunological method such as GC/MS or LC-MS/MS.
3. The test is designed for use with human urine only.
4. It is possible that other factors (eg, technical or procedural errors) and/or substances not listed in the specificity table may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance results obtained on a Beckman Coulter AU680 analyzer are shown below. The results obtained in your laboratory may differ from these data.

Precision:

Samples were prepared by spiking Oxazepam into drug free urine at the cutoff (100%), 25%, 50%, 75% and 100% above and below the cutoff and tested in both qualitative and semi-quantitative modes using a Clinical Laboratory and Standards Institute (CLSI) protocol. Results presented below were generated by testing all samples in replicates of 2, twice per day for 20 days, total n=80.

Qualitative Study Analysis

Spiked Concentration (ng/mL)	% of Cutoff (200 ng/mL)	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			# of Determinants	Immunoassay Results (Negative/Positive)
0	-100%	N/A	80	80/0
50	-75%	56.0	80	80/0
100	-50%	102.0	80	80/0
150	-25%	161.5	80	80/0
200	100%	214.0	80	16/64
250	+25%	255.5	80	0/80
300	+50%	299.0	80	0/80
350	+75%	348.0	80	0/80
400	+100%	403.0	80	0/80

Semi-Quantitative Study Analysis

Spiked Concentration (ng/mL)	% of Cutoff (200 ng/mL)	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			# of Determinants	Immunoassay Results (Negative/Positive)
0	-100%	N/A	80	80/0
50	-75%	56.0	80	80/0
100	-50%	102.0	80	80/0
150	-25%	161.5	80	80/0
200	100%	214.0	80	27/53
250	+25%	255.5	80	0/80
300	+50%	299.0	80	0/80
350	+75%	348.0	80	0/80
400	+100%	403.0	80	0/80

Accuracy

One hundred and six urine samples were tested by DRI Benzodiazepine assay in both qualitative and semi-quantitative modes. All samples were tested by LC-MS/MS. The overall concordance between LC-MS/MS and DRI Benzodiazepine assay was 96.2%.

Qualitative Accuracy study with LC-MS/MS as reference method

Candidate Device Results	Negative	<50% of cutoff concentration by LC/MS (<100 ng/mL)	Near cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (100-199 ng/mL)	Near cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (200-300 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>300 ng/mL)
Positive	0	1*	3*	5	45
Negative	48	2	2	0	0

Semi-quantitative Accuracy study with LC-MS/MS as reference method

Candidate Device Results	Negative	<50% of cutoff concentration by LC/MS (<100 ng/mL)	Near cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (100-199 ng/mL)	Near cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (200-300 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>300 ng/mL)
Positive	0	1*	3*	5	45
Negative	48	2	2	0	0

*** Discordant Result Table for Discrepant Samples near cutoff**

Sample ID	EIA		LC-MS/MS
	Qualitative Mode	Semi-quantitative Mode	Total Benzodiazepine Parent Only (ng/mL)
CA160606-045	Positive	Positive	86.20
CA160926-057	Positive	Positive	175.08
CA170605-001	Positive	Positive	151.52
CA160908-003	Positive	Positive	192.87

These four samples are discordant because of the presence of Benzodiazepine metabolites.
Sample CA160606-045 contains 3154.59 ng/mL 7-Aminoclonazepam .
Sample CA160926-057 contains 13.46 ng/mL α-Hydroxyalprazolam and 410.69 ng/mL 7-Aminoclonazepam.
Sample CA170605-001 contains 1.43 ng/mL α-Hydroxyalprazolam and 560.37 ng/mL 7-Aminoclonazepam.
Sample CA160908-003 contains 96.27 ng/mL α-Hydroxyalprazolam.

Analytical Recovery and Dilution Linearity

To demonstrate the dilution linearity for purposes of sample dilution and quality control of the entire assay range, drug free urine was spiked to the high calibrator level using Oxazepam (1000 ng/mL) and diluted with drug free urine to generate 10 intermediate levels. Each sample was run in replicates of 5 in semi-quantitative mode and the average was used to determine percent recovery compared to the expected target value.

Target Value (ng/mL)	Observed Value (ng/mL) n=5	Recovery (%)
0	-1.0	N/A
100	104.5	104.5
200	196.2	98.1
300	314.7	104.9
400	455.3	113.8
500	565.2	113.0
600	661.0	110.2
700	764.7	109.2
800	872.1	109.0
900	937.3	104.1
1000	1024.9	102.5

Specificity

The cross reactivity of benzodiazepine compounds and their metabolites was evaluated by adding known amounts of each compound to drug-free negative urine.

Cross reactivity of benzodiazepine compounds and structurally unrelated compound*

Structurally related and unrelated compounds	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
α-Hydroxyalprazolam	110	Positive	182
α-Hydroxytriazolam	140	Positive	143
Alprazolam	110	Positive	182
7-Aminoclonazepam	2,500	Positive	8
7-Aminoflunitrazepam	300	Positive	67
7-Aminonitrazepam	300	Positive	67
Bromazepam	170	Positive	118
Chlordiazepoxide	700	Positive	29
Clobazam	150	Positive	133
Clonazepam	210	Positive	95
Clorazepate	135	Positive	148
Delorazepam	150	Positive	133
Demoxepam	220	Positive	91
Desalkylflurazepam	130	Positive	154
Diazepam	110	Positive	182
Estazolam	100	Positive	200
Flunitrazepam	120	Positive	167
Flurazepam	150	Positive	133
2-Hydroxyethylflurazepam	120	Positive	167
Lorazepam	700	Positive	29
Lorazepam glucuronide	50,000	Negative	<0.4
Lormetazepam	275	Positive	73
Medazepam	325	Positive	62
Midazolam	180	Positive	111
Nitrazepam	130	Positive	154
Norchlordiazepoxide	800	Positive	25
Nordiazepam	110	Positive	182
*Oxaprozin	125,000	Positive	0.16
Oxazepam	200	Positive	100
Oxazepam glucuronide	50,000	Positive	0.4
Prazepam	200	Positive	100
Temazepam	160	Positive	125
Temazepam glucuronide	50,000	Negative	<0.4
Triazolam	130	Positive	154

Structurally unrelated compounds were evaluated by adding each substance to Oxazepam spiked at low (150 ng/mL) and high (250 ng/mL) controls at the concentrations indicated. As shown in the table below, the Controls were detected accurately, Low Control as Negative and the High Control as Positive, indicating that all the compounds evaluated exhibited minimal cross-reactivity at the concentrations tested.

Structurally unrelated compounds spiked at the concentration listed below into Low and High controls

Cross Reactants	Tested Concentration (ng/mL)	Low Control	High Control
6-Acetyl morphine	100,000	Negative	Positive
10,11 Dihydrocarbamazepine	100,000	Negative	Positive
11-nor-Δ9-THC-COOH	100,000	Negative	Positive
Acetaminophen	1,000,000	Negative	Positive
Acetylsalicylic acid	1,000,000	Negative	Positive
Amitriptyline	100,000	Negative	Positive
Amoxicillin	100,000	Negative	Positive
Amphetamine	100,000	Negative	Positive
Amisulpride	100,000	Negative	Positive
Benzotropine Mesylate	100,000	Negative	Positive
Benzoylecgonine	100,000	Negative	Positive

Table Continued

Cross Reactants	Tested Concentration (ng/mL)	Low Control	High Control
Brompheniramine	100,000	Negative	Positive
Buprenorphine	100,000	Negative	Positive
Caffeine	100,000	Negative	Positive
Captopril	100,000	Negative	Positive
Chlorpromazine	100,000	Negative	Positive
Chloroquine	100,000	Negative	Positive
Cimetidine	100,000	Negative	Positive
Clomipramine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Desipramine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
Diphenhydramine	500,000	Negative	Positive
Doxepine HCl	100,000	Negative	Positive
EDDP	100,000	Negative	Positive
EMDP	25,000	Negative	Positive
Enalapril	100,000	Negative	Positive
Fentanyl	100,000	Negative	Positive
Fluoxetine	500,000	Negative	Positive
Fluophenazine	100,000	Negative	Positive
Haloperidol	100,000	Negative	Positive
Heroin	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
Hydroxychloroquine	100,000	Negative	Positive
Hydroxyzine	100,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Imipramine	100,000	Negative	Positive
LAAM	100,000	Negative	Positive
Levorphanol	100,000	Negative	Positive
Levothyroxine	100,000	Negative	Positive
Maprotiline	100,000	Negative	Positive
Meperidine	100,000	Negative	Positive
Methadone	100,000	Negative	Positive
Methamphentamine	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3β-D-glucuronide	100,000	Negative	Positive
Morphine-6β-D-glucuronide	100,000	Negative	Positive
Nalbuphine	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Nifedipine	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Norhydrocodone	100,000	Negative	Positive
Norfluoxetine	500,000	Negative	Positive
Noroxycodone	100,000	Negative	Positive
Noroxymorphone	100,000	Negative	Positive
Norpropoxyphene	100,000	Negative	Positive
Norsertraline	62,500	Negative	Positive
Nortryptiline	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
Paroxetine	100,000	Negative	Positive

Table Continued

Cross Reactants	Tested Concentration (ng/mL)	Low Control	High Control
Perphenazine	100,000	Negative	Positive
Phencyclidine	100,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Procyclidine	100,000	Negative	Positive
Propoxyphene	100,000	Negative	Positive
Protriptyline	100,000	Negative	Positive
Ranitidine	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Sertraline	62,500	Negative	Positive
Sulpiride	100,000	Negative	Positive
Tapentadol	100,000	Negative	Positive
Thioridazine	100,000	Negative	Positive
Tramadol	100,000	Negative	Positive
Triprolidine	100,000	Negative	Positive
Verapamil	100,000	Negative	Positive
Zaleplon	100,000	Negative	Positive
Zolpidem	100,000	Negative	Positive
Zopiclone	100,000	Negative	Positive

Interference

The potential interference of pH and endogenous physiologic substances on recovery of Oxazepam using DRI Benzodiazepine assay was assessed by spiking known compounds of potentially interfering substances into the low (150 ng/mL) and high (250 ng/mL) controls urine for 200 ng/mL cutoff. In the presence of the compounds listed below, the controls were detected accurately, indicating that these compounds did not show interference in the assay.

Compound	Tested Concentration (mg/dL)	Low Control	High Control
Acetone	500	Negative	Positive
Ascorbic acid	150	Negative	Positive
Creatinine	400	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	5	Negative	Positive
Glucose	1000	Negative	Positive
Hemoglobin	150	Negative	Positive
Human serum albumin	200	Negative	Positive
Oxalic acid	50	Negative	Positive
Riboflavin	3	Negative	Positive
Sodium Chloride	1000	Negative	Positive
Urea	1000	Negative	Positive
pH	3.0	Negative	Positive
pH	4.0	Negative	Positive
pH	5.0	Negative	Positive
pH	6.0	Negative	Positive
pH	7.0	Negative	Positive
pH	8.0	Negative	Positive
pH	9.0	Negative	Positive
pH	10.0	Negative	Positive
pH	11.0	Negative	Positive

Specific Gravity

Drug free urine samples with specific gravity ranging in value from 1.004 to 1.029 were split and spiked to a final concentration of either 150 ng/mL or 250 ng/mL (the low and high control concentrations, respectively). These samples were then evaluated in qualitative and semi-quantitative modes. The Controls were detected accurately, indicating that no interference was observed.

Specific Gravity	Low Control	High Control
1.004	Negative	Positive
1.005	Negative	Positive
1.007	Negative	Positive
1.010	Negative	Positive
1.011	Negative	Positive
1.013	Negative	Positive
1.019	Negative	Positive
1.023	Negative	Positive
1.025	Negative	Positive
1.029	Negative	Positive

References

1. Mandatory Guideline for Federal Workplace Drug Testing Programs. National Institute on Drug Abuse. Federal Register Vol. 73, No. 228; 2008:71893
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3. Rubenstein KE, Schneider RS, and EF Ullman: Homogeneous enzyme immunoassay: A new immunochemical technique.Biochem Biophys Res Commun 47:846-854 (1972)
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5. Zaitsu K, Miki A, Katagi M, Tsuchihashi H. Long-term stability of various drugs and metabolites in urine, and preventive measures against their decomposition with special attention to filtration sterilization. *Forensic Science Intl* 174 (2008) 189-196.
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7. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).

Glossary:

http://www.thermofisher.com/symbols-glossary

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