DRI® Benzodiazepine Assay

Catalog No.: 0039 (100 mL Kit)
0040 (500 mL Kit)

Intended Use
The DRI® Benzodiazepine Assay is 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 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 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 dehydrogenase (G6PDH) 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):
Catalog No. 1664 DRI Negative Calibrator, 10 mL
1388 DRI Negative Calibrator, 25 mL
1588 DRI MultiDrug Urine Calibrator 1, 10 mL
1589 DRI MultiDrug Urine Calibrator 1, 25 mL
1591 DRI MultiDrug Urine Calibrator 2, 10 mL
1592 DRI MultiDrug Urine Calibrator 2, 25 mL
1594 DRI MultiDrug Urine Calibrator 3, 10 mL
1595 DRI MultiDrug Urine Calibrator 3, 25 mL
1597 DRI MultiDrug Urine Calibrator 4, 10 mL
1598 DRI MultiDrug Urine Calibrator 4, 25 mL
100200 MGC Primary DAU Control Set

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 metal azides. When disposing of such reagents, always flush with a large volume of water to prevent azide build-up.
3. 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.

Before performing the assay, refer to the analyzer-specific protocol sheet, which contains parameters and/or additional instructions for use.

Quality Control and Calibration
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.

Qualitative analysis
For qualitative analysis of samples, use the 200 ng/mL calibrator as a cutoff level. The DRI® MultiDrug 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.

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

Typical performance results obtained on a Hitachi 717 analyzer are shown below.4

**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.

<table>
<thead>
<tr>
<th>Calibrator/ Control</th>
<th>Within-run Precision</th>
<th>Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mA/min)</td>
<td>(mA/min)</td>
</tr>
<tr>
<td>150 ng/mL</td>
<td>375</td>
<td>375</td>
</tr>
<tr>
<td>200 ng/mL</td>
<td>406</td>
<td>406</td>
</tr>
<tr>
<td>250 ng/mL</td>
<td>434</td>
<td>434</td>
</tr>
</tbody>
</table>

Sensitivity

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

Specificity

Various benzodiazepine compounds were tested for cross-reactivity with the assay. The concentrations listed below are the lowest levels that yield positive results.

![Table showing the concentrations of various compounds tested in the assay.](image)

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%.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg/mL)</th>
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<tbody>
<tr>
<td>Acetaminophen</td>
<td>1000</td>
</tr>
<tr>
<td>Acetylpiracetamyl</td>
<td>1000</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>1000</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1000</td>
</tr>
<tr>
<td>Codeine</td>
<td>1000</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>1000</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>500</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>500</td>
</tr>
<tr>
<td>Methadone</td>
<td>1000</td>
</tr>
<tr>
<td>Morphine</td>
<td>200</td>
</tr>
<tr>
<td>Nor-Fluoxetine</td>
<td>500</td>
</tr>
<tr>
<td>Nor-Serozatine</td>
<td>1000</td>
</tr>
<tr>
<td>Oxaprozin</td>
<td>50</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>500</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>1000</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>1000</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>1000</td>
</tr>
<tr>
<td>Sertraline</td>
<td>500</td>
</tr>
</tbody>
</table>

DRI Benzodiazepine Assay

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Commercial EIA</td>
<td>42</td>
<td>7*</td>
</tr>
<tr>
<td>GC/MS</td>
<td>1*</td>
<td>50</td>
</tr>
</tbody>
</table>

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.

† 3 samples contained benzodiazepine compounds below the detection level by DRI Benzodiazepine Assay.

† Sample quantity not sufficient for further analysis.
References


Explanations of Symbols:

- CE Marking of Conformity
- Consult Instructions for Use
- European Community Authorized Representative
- Lot Number
- Catalog Number
- Temperature Limitation
- Use By
- Caution
- Manufacturer
- Biological risk
- For In Vitro Diagnostic Use
- Contents

Manufacturer:
Microgenics Corporation
46360 Fremont Blvd.
Fremont, CA 94538 USA
US Toll Free: 1-800-232-3342

Authorized Representative in E.U.:
Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau Germany
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10

Other countries:
Please contact your local Microgenics representative.

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