DRI® Barbiturate Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

REF 10015648 (3 x 18 mL Kit)
0225 (100 mL Kit)
0226 (500 mL Kit)

Intended Use
The DRI Barbiturate Assay is intended for the qualitative and semiquantitative determination of barbiturates 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
Drug abusers may abuse various barbiturates, such as short-acting secobarbital and long-acting phenobarbital, through oral ingestion or by intravenous and/or intramuscular injection. Long-term abuse can lead to respiratory depression or, in severe cases, coma. When ingested, a barbiturate is rapidly metabolized and excreted into urine, allowing immunoassays to detect recent use.

The DRI® Barbiturate Assay is a homogeneous enzyme immunoassay[3] using ready-to-use liquid reagents. The assay uses monoclonal antibodies that detect most barbiturates 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 drug from the sample, the G6PDH 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 monoclonal anti-barbiturate antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as preservative.
Enzyme Conjugate Reagent.
Contains barbiturate labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as 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 Calibrator 1, 10 mL
1589	DRI Multi-Drug Calibrator 1, 25 mL
1591	DRI Multi-Drug Calibrator 2, 10 mL
1592	DRI Multi-Drug Calibrator 2, 25 mL
1594	DRI Multi-Drug Calibrator 3, 10 mL
1595	DRI Multi-Drug Calibrator 3, 25 mL
1597	DRI Multi-Drug Calibrator 4, 10 mL
1598	DRI Multi-Drug 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.

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 for use. No reagent preparation is required. All assay components, when stored 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 secobarbital, is used as a cutoff reference for distinguishing "positive" from "negative" samples. In certain applications, 300 ng/mL has been used as a cutoff calibrator.

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.

Limitations
1. A positive result from this assay indicates only the presence of barbiturates and does not necessarily correlate to 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 data results obtained on the Hitachi 717 analyzer are shown below.[4] The results obtained in your laboratory may differ from these data.

Precision
The Negative, 200 ng/mL calibrator, 1000 ng/mL calibrator, Control 1 and Control 2 were assayed, and the following results were obtained:

Qualitative

Calibrator	Within-run (n=20)		Run-to-run (n=17)	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
0	147 ± 1.4	0.9	147 ± 0.7	0.5
200	239 ± 1.2	0.5	235 ± 0.8	0.3
1000	340 ± 2.1	0.6	332 ± 1.5	0.5

Semi-quantitative

Control	Within-run (n=20)		Run-to-run (n=17)	
	Mean ± SD (ng/mL)	% CV	Mean ± SD (ng/mL)	% CV
Control 1	157 ± 1.4	0.9	161 ± 2.2	1.4
Control 2	264 ± 1.2	0.8	264 ± 3.3	1.3

Sensitivity
Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 25 ng/mL on the Hitachi 717.

Accuracy
One hundred clinical urine specimens were tested with a commercially available EIA assay and DRI Barbiturate Assay. There was 100% agreement between the two methods. Seventy-eight samples were positive and twenty-two were negative by both assays. In addition, all seventy-eight positive samples were confirmed positive by the GC/MS method.

Specificity
Various potentially interfering substances were tested for cross-reactivity with the assay. The compounds listed in the table below produced a result approximately equivalent to the cutoff calibrator.

Compound	Concentrations Tested (ng/mL)
Alphenal	250
Amobarbital	200
Aprobarbital	200
Barbital	1500
Butabarbital	250
Butalbital	300
Butethal	300
Diallybarbital	600
Pentobarbital	500
Phenobarbital	600
Secobarbital	200
Talbutal	60
Thiopental	600

The compounds listed in the table below produced a negative result relative to the cutoff calibrator.

Compound	Concentrations Tested (ng/mL)
Acetaminophen	1000
Acetylsalicylic acid	1000
d-Amphetamine	1000
Benzoylecgonine	1000
Caffeine	100
Codeine	1000
Hydroxphenytoin (HPPH)	500
Meperidine	1000
Methadone	1000
Methaqualone	1000
Morphine	1000
Oxazepam	500
Phencyclidine	1000
Phenytoin (DPH)	500
Propoxyphene	1000

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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