# DRI® Methadone Assay

microgenics

For In Vitro Diagnostic Use

**Catalog No.** 0596 (100 mL Kit)
0597 (500 mL Kit)

## Intended Use

The DRI® Methadone Assay is intended for the qualitative and semiquantitative determination of methadone in human urine.

***The 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

Methadone, a synthetic opioid, has been used in the treatment of heroin addiction. Methadone compliance is essential and can be effectively monitored by urine screening for methadone or its metabolite.

When methadone is ingested, it is rapidly metabolized in the liver. The primary methadone metabolite is formed by N-demethylation to normethadone. However, normethadone is rarely detected as it readily dehydrates to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, commonly known as EDDP.[3,4] Further demethylation of EDDP forms 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP) which is the secondary metabolite of methadone.

Various techniques including TLC, GC and immunoassays are available for methadone compliance monitoring.[5] Both TLC and GC methods[6] are laborious and subject to interference. Immunoassays can be easily performed with an automated clinical chemistry analyzer. Determination of the presence of methadone in urine with an immunoassay will make widespread testing for compliance possible.

The DRI Methadone Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.[7] The assay uses specific antibodies, which can detect methadone 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 specific antibody binds to the drug labeled with G6PDH and the enzyme activity is inhibited. This phenomenon creates a direct relationship between the drug concentration in the 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-methadone antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

**Enzyme Conjugate Reagent:** Contains methadone derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

***Additional Materials Required (sold separately):***

Catalog No.
1664 DRI Negative Calibrator, 10 mL
1388 DRI Negative Calibrator, 25 mL
1588 DRI MultiDrug Calibrator 1, 10 mL
1589 DRI MultiDrug Calibrator 1, 25 mL
1591 DRI MultiDrug Calibrator 2, 10 mL
1592 DRI MultiDrug Calibrator 2, 25 mL
1594 DRI MultiDrug Calibrator 3, 10 mL
1595 DRI MultiDrug Calibrator 3, 25 mL
1597 DRI MultiDrug Calibrator 4, 10 mL
1598 DRI MultiDrug Calibrator 4, 25 mL
1599 DRI MultiDrug Urine Control 1, 10 mL
1553 DRI MultiDrug Urine Control 1, 25 mL
1600 DRI MultiDrug Urine Control 2, 10 mL
1555 DRI MultiDrug Urine Control 2, 25 mL

## Precautions and Warning

1. This test is for in-vitro diagnostic use only. The components 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 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.

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 the established range. If results fall outside of the established range, assay results are invalid.

***Qualitative analysis***
For qualitative analysis of samples, use the 300 ng/mL calibrator as a cutoff level. The DRI® Calibrator 2, which contains 300 ng/mL methadone, is used as a cutoff reference for distinguishing “positive” and “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.

## Limitations

1. A positive result from this assay indicates only the presence of methadone and does not necessarily correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by an other nonimmunological method such as GC, GC/MS or TLC.
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 data results obtained on the Hitachi 717 analyzer are shown below.[8]

***Precision***

The Negative, 300 ng/mL calibrator and 1000 ng/mL calibrator were assayed, and the following results were obtained:

**Qualitative**

| | Within-run (n=20) | | Run-to-run (n=12) | |
|---|---|---|---|---|
| Calibrator | Mean ± SD (mA/min) | % CV | Mean ± SD (mA/min) | % CV |
| Negative | 258 ± 1.3 | 0.5 | 258 ± 1.3 | 0.5 |
| 300 ng/mL | 340 ± 2.4 | 0.7 | 340 ± 2.9 | 0.8 |
| 1000 ng/mL | 472 ± 2.4 | 0.5 | 472 ± 4.6 | 0.9 |

**Semiquantitative**

| | Within-run (n=20) | | Run-to-run (n=12) | |
|---|---|---|---|---|
| Control | Mean ± SD (ng/mL) | % CV | Mean ± SD (ng/mL) | % CV |
| Control 1 | 166 ± 4.5 | 2.7 | 166 ± 5.7 | 3.4 |
| Control 2 | 403 ± 6.8 | 1.7 | 406 ± 7.1 | 1.7 |

***Sensitivity***

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 10 ng/mL.

***Accuracy***

Ninety-six clinical urine specimens were tested with a commercially available methadone assay and DRI Methadone Assay. There was 100% agreement between the two methods. Forty-six samples were positive and fifty were negative by both assays. In addition, all forty-six positive samples were confirmed positive by the GC/MS method.

***Specificity***

Various potentially interfering substances were tested for cross-reactivity with the assay. Table 1 lists the compounds producing a positive result at the concentration listed. Table 2 lists the compounds producing a negative result at the concentration listed.

**Table 1**

| Compound | Concentration Tested (ng/mL) |
|---|---|
| Methadone | 300 |
| Methadol | 750 |

**Table 2**

| Compound | Concentration Tested (ng/mL) |
|---|---|
| 1-α-Acetylmethadol (LAAM) | 5000 |
| Acetaminophen | 1000000 |
| Acetylsalicylic acid | 1000000 |
| Amitriptyline | 50000 |
| Amphetamine | 1000000 |
| Benzoylecgonine | 400000 |
| Caffeine | 100000 |
| Carbamazepine | 20000 |
| Cocaine | 200000 |
| Codeine | 500000 |
| Dextromethorphan | 250000 |
| Diphenhydramine | 1000000 |
| Ephedrine | 1000000 |
| Imipramine | 50000 |
| Meperidine | 150000 |
| Methadone Metabolite (EDDP) | 10000 |
| Methadone Metabolite (EMDP) | 10000 |
| Morphine | 200000 |
| Nortriptyline | 50000 |
| Orphenadrine | 1000000 |
| Oxazepam | 500000 |
| Phencyclidine | 500000 |
| Phenobarbital | 1000000 |
| Phenytoin | 40000 |
| Primidone | 24000 |
| Promethazine | 100000 |
| Propoxyphene | 250000 |
| Secobarbital | 1000000 |
| Theophylline | 50000 |
| Valproic Acid | 150000 |
| Verapamil | 1000000 |

## Bibliography


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2. *Mandatory Guidelines for Federal Workplace Drug Testing Programs*. National Institute on Drug Abuse. Federal Register Vol. **53**, No 69 pp 11970 (1988).
3. Pohland A, Boaz HE and HR Sullivan. *Synthesis and Identification of Metabolites Resulting from the Biotransformation of d,l-Methadone in Man and in Rat*. J Med Chem **14**:194-197 (1971).
4. Baselt RC and LJ Casarett. *Urinary Excretion of Methadone in Man. Clin Pharm Therap* **13**:64-70 (1971).
5. Ferrara SD. *Comparison of GLC-EMIT Analysis for the Assay of Methadone and Its Metabolite in Urine*. Vet Hum Toxicology **21** (Suppl):169-172 (1979).
6. Roerig DL et al. *Radioimmunoassay Compared to Thin-Layer and Gas-Liquid Chromatography for Detecting Methadone in Human Urine*. Clin Chem **22**:1915-1918 (1976).
7. Rubenstein KE, Schneider RS, and EF Ullman: *Homogeneous Enzyme Immunoassay: a New Immunochemical Technique*. Biochem Biophys Res Commun **47**:846-851, 1972.
8. Data on file at Microgenics Corporation.



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