DRI® Ecstasy Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10014681 (3 x 18 mL)
100075 (100 mL Kit)
100076 (500 mL Kit)

Intended Use
The DRI® Ecstasy Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative or semiquantitative determination of ecstasy drugs in human urine. The assay provides a simple and rapid analytical screening procedure for detecting ecstasy drugs at a cutoff level of 500 ng/mL.

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
Ecstasy drugs represent a group of ring-substituted methylenedioxy analogues of amphetamine, including 3, 4-methylenedioxyamphetamine (MDA), 3, 4 methylenedioxymethamphetamine (MDMA) and 3, 4-methylenedioxyethylamphetamine (MDEA). They are central nervous system (CNS) stimulants popularly abused for their psychotropic effects and are listed by the U.S. Drug Enforcement Administration as Schedule I (no accepted medical application with great abuse potential). At low doses, both MDMA and MDA produce euphoria, increased self-awareness, and an increased sense of trust. At higher doses, they are thought to be hallucinogenic. Toxic effects are similar to those of other CNS stimulants and include anxiety, depression, tachycardia, elevated blood pressure, cardiac arrhythmias, pupil dilation, and sleep disorders.

The length of time following drug use for which a positive result may ocur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life and the drug user's age, weight, activity and diet. Within the body, MDMA is known to metabolize to MDA by N-demethylation. Urinary excretion accounts for 65% of the dose as parent drug and 7% as MDA within 3 days. Urinary MDMA concentrations following a 1.5 mg/kg oral dose may exceed 17 mg/L. Other urinary metabolites include mono- and dihydroxy-derivatives of MDMA and MDA, resulting from fission of the methylene bridge, which are eliminated as conjugates. The human metabolism of MDA has not been studied. Urine concentrations in fatal cases of up to 160 mg/L have been recorded and are indicative of excretion of substantial portions of unchanged drugs.[3]

The DRI Ecstasy assay utilizes liquid, ready-to-use reagents and calibrators.[4] The assay uses specific antibodies, which can detect ecstasy drugs in urine with minimal cross-reactivity to various amphetamine compounds. The assay is based oncompetition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme, and free drug from the urine sample for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH causing a decrease in enzyme activity. This phenomenon creates a direct relationship between drug concentration in urine and 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 (R1):
Contains monoclonal anti-MDMA antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

Enzyme Conjugate Reagent (R2):
Contains MDMA labeled with glucose-6-phosphatedehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Materials Required (but not provided):

REF	Kit Description
1664	DRI Negative Calibrator, 10 mL
1388	DRI Negative Calibrator, 25 mL
100082	DRI Ecstasy 250 ng/mL Calibrator, 10 mL
100081	DRI Ecstasy 500 ng/mL Calibrator, 10 mL
100080	DRI Ecstasy 750 ng/mL Calibrator, 10 mL
100079	DRI Ecstasy 1000 ng/mL Calibrator, 10 mL
100202	MGC Select DAU Control Set, 3 x 5 mL

⚠ Precautions and Warnings
DANGER: DRI Ecstasy Immunoassay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% drug-specific antibody.
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 is 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.

1. This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. 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.
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 refrigerated, are stable until the expiration date indicated on the label.

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 to 8°C for 7 days.[6] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C for 21 weeks.[5, 7]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[8]

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
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 for each analyzer for chemistry parameters before performing the assay.

Quality Control and Calibration[9]
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. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Qualitative analysis
For qualitative analysis of samples, use the 500 ng/mL calibrator as a cut off level.

Semiqualitative analysis
For semiquantitative analysis of samples, use all calibrators. All QC requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

Qualitative results

The 500 ng/mL calibrator is used as a Cutoff reference for distinguishing "positive" from "negative" samples. A sample that exhibits a change in absorbance value (ΔA) equal to or greater than that obtained with cutoff calibrator is considered positive. A sample, which exhibits a change in absorbance value (ΔA) lower than that 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 instrument parameter sheets for analyzer specific protocols.

Limitations

1. A positive result from this assay indicates only the presence of Ecstasy 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.
3. The test is designed for use with human urine only.
4. It is possible that other substances and/or factors, other than those investigated in the specificity study, may interfere with the test and cause false results e.g., technical, procedural issues or other Ecstasy-like compounds.

Typical Performance Characteristics

Performance results obtained on the Hitachi 717 analyzer are shown below.[9] The results obtained in your laboratory may differ from these data.

Precision

Negative control, positive control and cutoff calibrator were tested using a modified NCCLS protocol. The test was run in rate mode by testing all three levels in replicates of 6, twice per day for 10 days.

Hitachi 717 Qualitative (mA/min)

Using the 120 ng/mL cutoff calibrator	Within-run Precision		Total Precision	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Low Control (375 ng/mL)	254 ± 2.3	0.9	254 ± 7.0	2.8
Cutoff (500 ng/mL)	332 ± 3.4	1.0	332 ± 8.9	2.7
High Control (625 ng/mL)	395 ± 3.8	1.0	395 ± 7.2	1.8

Hitachi 717 Semi-quantitative (ng/mL)

Using the 120 ng/mL cutoff calibrator	Within-run Precision		Total Precision	
	Mean ± SD (ng/mL)	% CV	Mean ± SD (ng/mL)	% CV
Low Control (375 ng/mL)	359 ± 5.7	1.6	359 ± 9.1	2.5
Cutoff (500 ng/mL)	500 ± 6.9	1.4	500 ± 10.7	2.1
High Control (625 ng/mL)	630 ± 9.5	1.5	630 ± 13.7	2.2

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 22 ng/mL.

Accuracy

A total of ninety-two Ecstasy positive and eighteen Ecstasy negative clinical samples were tested with DRI Ecstasy Assay and compared to GC/MS. 100% agreement was obtained between the two methods. An additional forty previously screened negative samples also tested negative by DRI Ecstasy assay. The performance of DRI Ecstasy Assay compared to GC/MS is presented below.

Qualitative

DRI Ecstasy 500 ng/mL Cutoff	GC/MS 500 ng/mL Cutoff +	GC/MS 500 ng/mL Cutoff -
+	92	0
-	0	18

Semiquantitative

DRI Ecstasy 500 ng/mL Cutoff	GC/MS 500 ng/mL Cutoff +	GC/MS 500 ng/mL Cutoff -
+	92	0
-	0	18

Of the one hundred and ten total clinical samples confirmed by GC/MS, ten were between 375 ng/mL and 500 ng/mL (-25% of cut-off concentration) and ten samples were between 500 ng/mL and 625 ng/mL (+25% of cut-off concentration). Both positive and negative sample agreement was 100%.

Specificity

The specificity of the assay was evaluated by testing structurally related compounds and structurally unrelated but commonly used drugs.

Concentrations of compounds that produce a positive result approximately equivalent to 500 ng/mL MDMA cutoff are listed below. The percent cross-reactivities are also presented.

Structurally related compounds:

Compound	Concentration (ng/mL)	% Cross Reactivity
MDA	750	67
MDEA	460	109
MBDB	1700	29
BDB	900	56
para-Methoxyamphetamine (PMA)	4500	11
para-Methoxymethamphetamine (PMMA)	1500	33

Concentrations of compounds that produce a negative result below 500 ng/mL MDMA cutoff are listed below. The percent cross-reactivities are also presented.

Structurally related compounds:

Compound	Concentration (ng/mL)	% Cross Reactivity
d-Amphetamine	600,000	0.1
l-Amphetamine	90,000	0.6
d,l-Amphetamine	180,000	0.3
l-Ephedrine	800,000	0.1
d-Methamphetamine	600,000	0.1
l-Methamphetamine	62,000	0.8
d,l-Methamphetamine	100,000	0.5
Phentermine	220,000	0.2
d,l-Phenylpropanolamine	800,000	0.1
d-Pseudoephedrine	1,000,000	0.1

$$\% \text{ cross reactivity} = \frac{\text{cutoff conc.}}{\text{observed conc.}} \times 100$$

Structurally unrelated compounds that produce a negative result at 500 ng/mL cutoff:

Compound	Concentration (ng/mL)
Acetaminophen	1,000,000
Acetylsalicylic acid	1,000,000
Amoxicillin	1,000,000
Benzoylecgonine	1,000,000
Caffeine	100,000
Captopril	1,000,000
Chlordiazepoxide	250,000
Cimetidine	500,000
Codeine	1,000,000
Diazepam	1,000,000
Digoxin	100,000
Enalapril	1,000,000
Fluoxetine	500,000
Ibuprofen	1,000,000
Levothyroxine	100,000
Methadone	1,000,000
Morphine	1,000,000
Nifedipine	50,000
Phencyclidine	1,000,000
Phenobarbital	1,000,000
Propoxyphene	1,000,000
Ranitidine	250,000
Salicyluric acid	1,000,000
THC	10,000
Tolmetin	500,000
Verapamil	1,000,000

Interference

Endogenous and some exogenous substances were studied for their interference in the Ecstasy assay. No interference was observed in urine samples containing the compounds up to the concentrations listed below. The pH of the urine sample was also studied for possible interference.

Compound	Concentration
Acetone	1000 mg/dL
Ascorbic Acid	1500 mg/dL
Creatinine	500 mg/dL
Ethanol	1 %
Galactose	10 mg/dL
Glucose	3 g/dL
Hemoglobin	300 mg/dL
Human Serum Albumin	500 mg/dL
Oxalic Acid	100 mg/dL
Riboflavin	7.5 mg/dL
Sodium Chloride	6 g/dL
Urea	6 g/dL
pH Range	3-11

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 Programs". National Institute on Drug Abuse. Federal Register Vol. 53, No 69, pp11970 (1988).
3. Baselt RC and Cravey RH. "Disposition of Toxic Drugs and Chemicals in Man". Chemical Toxicology Institute, 4th ed, Foster City, Calif., (1995).
4. Rubenstein KE, Schneider RS, and EF Ullman. "Homogeneous Enzyme Immunoassay: A New Immunochemical Technique". Biochem. Biophys. Res. Commun. 47, 846, (1972).
5. Clauwaert KM, Van Bocxlaer FJ, De Leenheer AP. Stability study of the designer drugs "MDA, MDMD and MDEA" in water, serum, whole blood, and urine under various storage temperatures. *Forensic Science International* 124 (2001) 36-42.
6. Cao Z, Kaleta E, Wang P. Simultaneous Quantitation of 78 Drugs and Metabolites in Urine with a Dilute-And-/shoo LC-MS-MS Assay. *Journal of Analytical Toxicology* 2015,:39:355-346.
7. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
8. *Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program: Final Guidelines; Federal Register,* Substance Abuse and Mental Health Administration (SAMHSA), (1994) 110 (June 9):11983.
9. Data on file at Microgenics, a part of Thermo Fisher Scientific.

Glossary:

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

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