Emit® II Plus Benzodiazepine Assay

2019-11 10871437_K

See shaded sections:
Updated information from 2017-03 version.

Catalog Number	Product Description	Quantity/ Volume
OSR9F229	**Emit® II Plus Benzodiazepine Assay**	
	OSR9F618 R1 (Antibody/Substrate Reagent 1)	2 x 31 mL
	OSR9F648 R2 (Enzyme Reagent 2)	2 x 15 mL
9A509UL	**Emit® Calibrator/Control Level 0***	1 x 14 mL
9A549UL	**Emit® Calibrator/Control Level 2 (100)***	1 x 14 mL
9A569UL	**Emit® Calibrator/Control Level 3 (200)***	1 x 14 mL
9A589UL	**Emit® Calibrator/Control Level 4 (300)***	1 x 14 mL
9A609UL	**Emit® Calibrator/Control Level 5 (1000)***	1 x 14 mL

Required for calibrating the Emit® II Plus Benzodiazepine Assay. Sold separately. To determine the appropriate calibrators required for use, see Table 1.

Note: Reagents and calibrators/controls are shipped ready to use in liquid form. No reconstitution is required.

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

Note: These reagents are qualified for use with these calibrators only. However, other material may be used for quality control purposes.

Table 1 — Emit® Calibrators/Controls for Use with the Emit® II Plus Benzodiazepine Assay

	Qualitative Analysis		Semiquantitative Analysis	
Desired Cutoff Level (ng/mL)	Required Cal/Control Level	Concentration of Lormetazepam (ng/mL)	Required Cal/Control Level	Concentration of Lormetazepam (ng/mL)
	Level 0	0		
200	Level 3	200	Level 0	0
	Level 5	1000	Level 2	100
			Level 3	200
	Level 0	0	Level 4	300
300	Level 4	300	Level 5	1000
	Level 5	1000		

Note: The Emit® Calibrators/Controls contain the stated concentrations of lormetazepam listed in Table 1. Levels 2, 3, 4, and 5 contain additional drugs of abuse that do not affect the assay. See the Emit ® Calibrators/Controls instructions for use. For any individual cutoff level, a calibrator/ control is used either as a calibrator or as a control when the assay is used for qualitative analysis. When a calibrator/control is used as a calibrator for an individual cutoff level, the other level calibrators/controls (above or below it, as listed above) are used as controls.

1 INTENDED USE

The Emit® II Plus Benzodiazepine Assay is a homogeneous enzyme immunoassay with a 200 ng/mL or 300 ng/mL cutoff. The assay is intended for use in the qualitative and semiquantitative analyses of benzodiazepines in human urine. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

The Emit® II Plus Benzodiazepine 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) is the preferred confirmatory method.[1] Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 SUMMARY

Benzodiazepines are sedative-hypnotic drugs that are structurally similar and include widely used drugs such as chlordiazepoxide, diazepam, and oxazepam. The different benzodiazepines are absorbed at different rates, and the timing of their psychoactive effects varies with the absorption rate. Benzodiazepines are usually taken orally and are metabolized in the liver. Some benzodiazepine metabolites are pharmacologically active.[2] Benzodiazepines potentiate the effect of other central nervous system depressants, such as ethyl alcohol.[3]

The Emit® II Plus Benzodiazepine Assay, an enzyme immunoassay technique, tests for benzodiazepines and their metabolites in human urine. Positive results for specimens containing other compounds structurally unrelated to benzodiazepines have not been observed. The cutoff levels for distinguishing positive from negative specimens are 200 ng/mL and 300 ng/mL.

Methods historically used for detecting benzodiazepines in biological fluids include gas chromatography with electron-capture[4] or flame-ionization detection,[5] high-performance liquid chromatography,[6] thin-layer chromatography,[7] fluorescence-TLC densitometry,[8] enzyme immunoassay,[9] and radioimmunoassay.[10]

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.[1]

3 METHODOLOGY

The Emit® II Plus Benzodiazepine Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[11] The assay is based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 REAGENTS

Reagents contain the following substances:

Sheep polyclonal antibodies to diazepam (1.2 µg/mL), glucose-6-phosphate (5.5 mM), nicotinamide adenine dinucleotide (3.5 mM), bovine serum albumin, diazepam labeled with G6PDH (0.62 U/mL), Tris buffer, preservatives, and stabilizers.

Risk and Safety:

H317
P280, P272, P302 + P352, P333 + P313, P501
Warning!
May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

Safety data sheets (MSDS/SDS) available on siemens.com/healthcare

Precautions

- For *in vitro* diagnostic use.
- Reagent 1 contains nonsterile sheep antibodies.
- Reagent 2 contains nonsterile mouse antibodies.
- Reagents 1 and 2 contain nonsterile bovine serum albumin.
- Do not use after expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents

The Emit® II Plus Benzodiazepine Assay reagents are provided ready to use; no preparation is necessary.

Storage of Assay Components

- Improper storage of reagents can affect assay performance.
- When not in use, store reagents upright at 2–8°C and with screw caps tightly closed.
- Unopened reagents are stable until the expiration date printed on the label, if stored upright at 2–8°C.
- Do not freeze reagents or expose them to temperatures above 32°C.

5 SPECIMEN COLLECTION AND PREPARATION

- Urine specimens may be collected in plastic (ie, polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics can adsorb certain drugs.
- Internal testing has shown that, if not analyzed immediately, specimens may be stored unrefrigerated for up to 7 days. Specimens may be stored refrigerated for 30 days before analysis. After 7 days unrefrigerated or 30 days refrigerated, samples should be stored frozen.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Specimens with high turbidity should be centrifuged before analysis.
- The recommended pH range for urine specimens is 3.0–11.0.
- Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.
- Human urine specimens should be handled and treated as if they were potentially infectious.

6 PROCEDURE

Materials Provided

Emit® II Plus Benzodiazepine Assay
Reagent 1
Reagent 2

Materials Required But Not Provided

Emit® Calibrators/Controls
Commercially available controls (see Quality Control, Semiquantitative Analysis)

Refer to the instrument User's Guide for appropriate instrument checks and maintenance instructions.

Calibration

Qualitative Analysis

Run the appropriate Emit® Calibrator/Control—Level 3 (200 ng/mL Cutoff), or Level 4 (300 ng/mL Cutoff)—in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the instrument User's Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis

Prepare a calibration curve by running a reagent blank (blue rack) and Emit® Calibrators/Controls Level 2 (100 ng/mL), Level 3 (200 ng/mL), Level 4 (300 ng/mL), and Level 5 (1000 ng/mL). Validate the calibration by running controls (see Quality Control). Refer to the instrument User's Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

Validate the calibration by assaying controls. Ensure that the result from the Emit® Calibrator/Control level (Level 0 [0 ng/mL] or Level 5 [1000 ng/mL]) relates appropriately to the cutoff calibrator result from the selected cutoff calibrator level (Level 3 [200 ng/mL] or Level 4 [300 ng/mL]). Once calibration is validated, run urine specimens.

Semiquantitative Analysis

For a selected cutoff level (200 ng/mL or 300 ng/mL), validate the calibration curve by assaying commercial controls. Ensure that control results fall within acceptable limits as defined by your laboratory. Once the calibration curve is validated, run urine specimens.

Qualitative and Semiquantitative Analysis

1. Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of Quality Control (QC) material with known Benzodiazepine concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
2. Refer to the instrument operator's manual for appropriate instrument checks.

Evaluation and Interpretation of Results

When the Emit® II Plus Benzodiazepine Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given specimen cannot be estimated. The assay results distinguish between positive and negative specimens—positive indicating specimens contain benzodiazepines; negative indicating specimens do not contain benzodiazepines, or benzodiazepines are present in concentrations below the cutoff level for this assay.

- A specimen that gives a change in rate value equal to or higher than the rate of the selected cutoff calibrator level is interpreted as positive.
- A specimen that gives a change in rate value lower than the rate of the selected cutoff calibrator level is interpreted as negative.

When used semiquantitatively, the Emit® II Plus Benzodiazepine Assay yields approximate, cumulative concentrations of the drugs detected by the assay (See Section 8, Specific Performance Characteristics, Analytical Recovery). The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for confirmation by GC/MS. Semiquantitation also permits the laboratory to establish quality control procedures and assess control performance.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

7 LIMITATIONS OF THE PROCEDURE

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of benzodiazepines.
- Boric acid is not recommended as a preservative for urine.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- Immunoassays that produce a single result in the presence of a drug and its metabolites cannot fully quantitate the concentration of individual components.
- The glucuronide metabolite of α-Hydroxyalprazolam cross-reacts with this assay. Other glucuronide metabolites such as Lorazepam, Oxazepam, and Temazepam cross-react to a limited extent. The cross-reactivity of other glucuronide metabolites with this assay is not known.
- Therapeutic doses of oxaprozin (DAYPRO), a non-benzodiazepine, may produce positive results with this assay. A positive result from an individual taking oxaprozin should be interpreted with caution and confirmed by another method.

8 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® II Plus Benzodiazepine Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Positive specimens were confirmed by GC/MS. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to refer only to reagents.

Precision

Within-run precision was determined by assaying 2 replicates of each cutoff calibrator/control and positive and negative controls twice a day for 20 days (N=80). Total precision was also calculated from these data. Table 2 summarizes the findings at the 200 ng/mL cutoff; Table 3 summarizes the findings at the 300 ng/mL cutoff.

Table 2 — Within-Run and Total Precision at 200 ng/mL

	Within-Run Precision			Total Precision		
Benzodiazepine 150 ng/mL	**Cutoff Cal**	**Control 75%**	**Control 125%**	**Cutoff Cal**	**Control 75%**	**Control 125%**
Mean	384	364	398	384	364	399
SD	3.0	1.8	2.1	3.9	2.6	3.5
%CV	0.8	0.5	0.5	1.0	0.7	0.9

Table 3 — Within-Run and Total Precision at 300 ng/mL

	Within-Run Precision			Total Precision		
Benzodiazepine 300 ng/mL	**Cutoff Cal**	**Control 75%**	**Control 125%**	**Cutoff Cal**	**Control 75%**	**Control 125%**
Mean	413	391	428	413	391	428
SD	2.1	8.0	2.0	3.8	8.5	3.9
%CV	0.5	2.1	0.5	0.9	2.2	0.9

Comparative Analysis

Clinical urine specimens were analyzed on the AU400/AU600 Clinical Chemistry System and on the SYVA®-30R Biochemical System. Specimens positive by either method contained oxazepam ranging from 58 to 13174 ng/mL. Table 4 summarizes the number of positive/negative results identified and the percent agreement with the SYVA®-30R Biochemical System.

Table 4 — Summary of Comparative Analysis

Assay	Positive	Negative	% Agreement
Benzodiazepine 200	80	50	99
Benzodiazepine 300	61	72	93

The use of lormetazepam as the Calibrator/Control analyte increases the apparent sensitivity of the Emit® II Plus Benzodiazepine Assay compared to the use of oxazepam in the Emit® II Benzodiazepine Assay.

Analytical Recovery

Negative human urine specimens were spiked with known concentrations of lormetazepam. Qualitative analysis of the specimens spiked with drug concentrations lower than the cutoff concentration were correctly identified as negative 100% of the time. Specimens spiked with drug concentrations greater than the cutoff were correctly identified as positive 100% of the time. Results from semiquantitative analysis of the specimens are listed below.

Table 5 — Analytical Recovery of Lormetazepam-Spiked Samples

Concentration (ng/mL)	Mean (ng/mL)
100	86
140	134
260	255
500	594
950	966

Specificity

The Emit® II Plus Benzodiazepine Assay detects benzodiazepines and benzodiazepine metabolites in human urine.

Table 6 lists the concentrations of compounds that produce a result that is approximately equivalent to the 200 ng/mL and 300 ng/mL calibrator/control cutoffs, respectively. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. These concentrations are within the range of the levels found in urine following use of the drug or, in the case of metabolites, the parent compound. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 6 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 6 — Concentrations (ng/mL) of Benzodiazepine Compounds That Produce a Result Approximately Equivalent to the 200 ng/mL and 300 ng/mL Lormetazepam Cutoffs

Compound	Concentration (ng/mL) at 200 ng/mL Cutoff	Concentration (ng/mL) at 300 ng/mL Cutoff
Alprazolam	65	79
7-Aminoclonazepam	5300	8600
7-Aminoflunitrazepam	930	1400
Bromazepam	630	1400
Chlordiazepoxide	3300	7800
Clobazam	260	230
Clonazepam	210	320
Clorazepate	*	*
Clotiazepam	380	670
Demoxepam	1600	4000
N-Desalkylflurazepam	130	160
N-Desmethyldiazepam	110	140
Diazepam	70	120
Estazolam	90	110
Flunitrazepam	140	160
Flurazepam	190	250
Halazepam	110	160
α-Hydroxyalprazolam	100	150
α-Hydroxyalprazolam glucuronide†	110	120
1-*N*-Hydroxyethylflurazepam	150	150
α-Hydroxytriazolam	130	190
Ketazolam	100	140
Lorazepam	600	890
Lorazepam glucuronide†	>20000	>20000
Medazepam	150	210
Midazolam	130	160
Nitrazepam	78	130
Norchlordiazepoxide	4500	7500
Oxazepam	250	350
Oxazepam glucuronide†	>30000	>40000
Phenazepam	90	130
Prazepam	90	130
Temazepam	140	210
Temazepam glucuronide†	>20000	>20000
Tetrazepam	70	100
Triazolam	130	180

Clorazepate degrades rapidly in stomach acid to nordiazepam. Nordiazepam hydroxylates to oxazepam.

†See Section 7, Limitations of the Procedure.

Table 7 lists the compounds that produce a negative result by the Emit® II Plus Benzodiazepine Assay. Specificity testing was performed at the 200 ng/mL cutoff, which represents the greatest potential for cross-reactivity. Positive results for compounds structurally unrelated to benzodiazepines have not been observed.

Table 7 — Concentrations of Compounds Showing a Negative Response

Compound	Concentration Tested (µg/mL) at the 200 ng/mL (0.2 µg/mL) Cutoff
Acetaminophen	1000
α-Acetyl-*N,N*-dinormethadol (dinor LAAM)	25
L-α-Acetylmethadol (LAAM)	25
N-Acetylprocainamide (NAPA)	400
Acetylsalicylic acid	1000
Amitriptyline	1000
D-Amphetamine	1000
Benzoylecgonine	1000
Buprenorphine	1000
Caffeine	1000
Cimetidine	1000
Clomipramine	2.5
Clonidine	1000
Codeine	500
Cotinine	100
Cyclobenzaprine	1000
Desipramine	800
Diphenhydramine	1000
Doxepin	1000
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1000
Fluoxetine	1000
Glutethimide	500
Ibuprofen	1000
Ketamine	100
Ketorolac Tromethamine	1000
LSD	10 ng/mL
Meperidine	1000
D-Methamphetamine	35
Methaqualone	1500
Morphine	1000
Naproxen	1000
Nortriptyline	1000
Phencyclidine	1000
Phenytoin	1000
Promethazine	1000
Propoxyphene	1000
Ranitidine	1000
Scopolamine	500
Secobarbital	1000
11-nor-Δ^9-THC-9-COOH	100
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2 mg/mL
Zolpidem	100

Non-Interfering Substances

Each of the following compounds when added to urine at +/- 25% concentration of the cutoff do not yield a false response relative to the 200 ng/mL cutoff:

Table 8 — Non-Interfering Substances

Compound	Concentration
Acetone	1.0 g/dL
Ascorbic Acid	1.5 g/dL
Bilirubin	0.25 mg/dL
Creatinine	0.5 g/dL
Ethanol	1.0 g/dL
Gamma Globulin	0.5 g/dL
Glucose	2.0 g/dL
Hemoglobin	115 mg/dL
Human Serum Albumin	0.5 g/dL
Oxalic Acid	0.1 g/dL
Riboflavin	7.5 mg/dL
Sodium Chloride	3.0 g/dL
Urea	6.0 g/dL

Sensitivity

The senstivity level (minimum detection limit) of the Emit® II Plus Benzodiazepine Assay is 23 ng/mL. This level represents the lowest concentration of lormetazepam that can be distinguished from 0 ng/mL with a confidence level of 95%.

9 REFERENCES

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Symbols Key

	Do not reuse
	Use By
LOT	Batch Code
REF	Catalogue Number
	Caution, consult accompanying documents
	Manufacturer
EC REP	Authorized Representative in the European Community
	Contains sufficient for <n> tests
IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation
	Consult Instructions for Use
NON STERILE	Non-sterile
CE	CE Mark
CONTENTS	Contents
	Reconstitution Volume
LEVEL	Level

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