

Emit® II Plus Propoxyphene Assay

September 2010

9G052.2D_B

Catalog Number	Product Description	Quantity/Volume
OSR9G229	Emit® II Plus Propoxyphene Assay	
	OSR9G618 R1 (Antibody/Substrate Reagent 1)	2 x 27 mL
	OSR9G648 R2 (Enzyme Reagent 2)	2 x 13 mL
9A509UL	Emit® Calibrator/Control Level 0*	1 x 14 mL
9A549UL	Emit® Calibrator/Control Level 2 (150)*	1 x 14 mL
9A569UL	Emit® Calibrator/Control Level 3 (300)*	1 x 14 mL
9A589UL	Emit® Calibrator/Control Level 4 (500)*	1 x 14 mL
9A609UL	Emit® Calibrator/Control Level 5 (1000)*	1 x 14 mL

*Required for calibrating the Emit® II Plus Propoxyphene 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 in Qualitative or Semiquantitative Analysis

Qualitative Analysis		Semiquantitative Analysis	
Required Cal/Control Level	Concentration of Propoxyphene (ng/mL)	Required Cal/Control Level	Concentration of Propoxyphene (ng/mL)
Level 0	0	Level 0	0
Level 3	300	Level 2	150
Level 5	1000	Level 3	300
		Level 4	500
		Level 5	1000

Note: The Emit® Calibrators/Controls contain the stated concentrations of propoxyphene listed in Table 1. Emit® Calibrator/Control Levels 2, 3, 4, and 5 contain additional drugs of abuse that do not affect the assay. For any individual cutoff listed, 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. See the Emit® Calibrators/Controls instructions for use.

1 INTENDED USE

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

The Emit® II Plus Propoxyphene 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

Propoxyphene and propoxyphene napsylate, a propoxyphene salt, are mildly effective narcotic analgesics used to treat mild to moderate pain. Propoxyphene is structurally related to methadone and produces central nervous system effects similar to those of morphine-like opioids. When given orally, propoxyphene is one-half to two-thirds as potent as codeine and has a similar incidence of side effects, which include nausea, anorexia, constipation, abdominal pain, and drowsiness. A synergistic effect is produced when propoxyphene is given in combination with aspirin.²

Propoxyphene and propoxyphene napsylate may be toxic and even fatal at levels that exceed the recommended therapeutic dosages, particularly because they are metabolized quickly. In addition to respiratory depression, toxic doses may produce convulsions, delusions, hallucinations, confusion, cardiotoxicity, and pulmonary edema.²

The Emit® II Plus Propoxyphene Assay uses a cutoff of 300 ng/mL propoxyphene. The assay detects propoxyphene and propoxyphene salts, such as propoxyphene napsylate, in human urine (see Table 6). It also detects norpropoxyphene (*N*-desmethyldextropropoxyphene), the major urinary metabolite of propoxyphene. Positive results for specimens containing other compounds structurally unrelated to propoxyphene have not been observed.

Methods historically used for detecting propoxyphene in biological fluids include ultraviolet spectrophotometry, gas chromatography, and enzyme immunoassay.³

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 Propoxyphene Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.⁴ 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 specimen 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 propoxyphene (4.1 µg/mL), glucose-6-phosphate (5.5 mM), nicotinamide adenine dinucleotide (3.5 mM), bovine serum albumin, propoxyphene labeled with G6PDH (0.47 U/mL), Tris buffer, preservatives, and stabilizers.

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 Propoxyphene 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.
- If not analyzed immediately, specimens may be stored unrefrigerated for up to 7 days following collection. After 7 days, specimens 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 3.0–9.0.
- 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 Propoxyphene 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 Emit® Calibrator/Control Level 3 (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 Emit® Calibrators/Controls Level 0 (0 ng/mL), Level 2 (150 ng/mL), Level 3 (300 ng/mL), Level 4 (500 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 (Emit® Calibrator/Control Level 3 [300 ng/mL]) result. Once calibration is validated, run urine specimens.

Semiquantitative Analysis

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.

Evaluation and Interpretation of Results

When the Emit® II Plus Propoxyphene Assay is used as a qualitative assay, the amount of propoxyphene or propoxyphene metabolites or analogs detected by the assay in any given specimen cannot be estimated. The assay results distinguish between positive and negative specimens—positive indicating specimens contain propoxyphene or propoxyphene metabolites or analogs. Negative results may indicate specimens do not contain propoxyphene or propoxyphene metabolites or analogs, or propoxyphenes 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 cutoff calibrator level is interpreted as positive.
- A specimen that gives a change in rate value lower than the rate of the cutoff calibrator level is interpreted as negative.

When used semiquantitatively, the Emit® II Plus Propoxyphene Assay yields approximate, cumulative concentrations of the drugs detected by the assay (see Section 8, Specific Performance Characteristics, Analytical Recovery or Specificity).

7 LIMITATIONS OF THE PROCEDURE

- The assay is designed for use only with human urine.
- A positive result from the assay indicates only the presence of propoxyphene but does not indicate or measure intoxication.
- 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 concentrations of propoxyphene or propoxyphene metabolites or analogs in urine 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.

8 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® II Plus Propoxyphene 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. Tables 2 and 3 summarize the data, which are presented in mAU/min.

Table 2 — Within-Run Precision

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	541	490	580
SD	4.3	3.8	3.7
%CV	0.8	0.8	0.6

Table 3 — Total Precision

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	541	490	580
SD	7.9	6.4	5.2
%CV	1.5	1.3	0.9

Comparative Analysis

Clinical urine specimens were analyzed using Emit® II Plus Propoxyphene Assay on the AU400/AU600 Clinical Chemistry System and using Emit® II Propoxyphene Assay on the SYVA®-30R Biochemical System. Specimens positive by either method contained propoxyphene ranging from 404 ng/mL to 56662 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
Propoxyphene	48	52	99

Analytical Recovery

Negative human urine specimens were spiked with known concentrations of propoxyphene. Specimens spiked with drug concentrations lower than the cutoff concentration and tested qualitatively 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. Table 5 summarizes the results on semiquantitative analysis of the specimens.

Table 5 — Semiquantitative Analysis of Propoxyphene-Spiked Samples

Concentration (ng/mL)	Mean (ng/mL)
150	137
210	186
390	420
450	480
500	497

Specificity

The Emit® II Plus Propoxyphene Assay detects propoxyphene or propoxyphene metabolites or analogs in human urine.

Tables 6 and 7 list the compounds this assay detects and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator/Control Level 3, 300 ng/mL). 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 levels found in urine following use of the compound 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 Tables 6 and 7 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 6 — Concentration of the Compound That Produces a Result Approximately Equivalent to the 300 ng/mL Propoxyphene Cutoff

Compound	Concentration (ng/mL)
Norpropoxyphene	800

Table 7 — Concentrations (µg/mL) of Compounds That Produce a Result Approximately Equivalent to a 300 ng/mL Propoxyphene Cutoff

Compound	Concentration (ng/mL)
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	5000
Imipramine	30

Table 8 lists the concentrations of compounds that show a negative response to the Emit® II Plus Propoxyphene Assay at a 300 ng/mL cutoff level.

Table 8 — Concentrations of Compounds Showing a Negative Response

Compound	Concentration (µg/mL)
Acetaminophen	1000
α-Acetyl-N,N-dinormethadol (dinor LAAM)	25
L-α-Acetylmethadol (LAAM)	25
N-Acetylprocainamide (NAPA)	400
Acetylsalicylic acid	1000
Amitriptyline	125
D-Amphetamine	1000
Benzoylcegonine	1000
Buprenorphine	1000
Caffeine	1000
Cimetidine	1000
Clomipramine	2.5
Clonidine	1000
Codeine	500
Cotinine	100
Cyclobenzaprine	7.8
Desipramine	5
Diphenhydramine	1000
Doxepin	31
Doxylamine	500
Fluoxetine	125
Glutethimide	500
Ibuprofen	1000
Ketamine	100
Ketorolac Tromethamine	1000
Lormetazepam	1
LSD	10 ng/mL
Meperidine	1000
Methadone	100
D-Methamphetamine	35
Methaqualone	1500
Morphine	1000
Naproxen	1000
Nortriptyline	31
Oxazepam	300
Phenytoin	1000
Promethazine	125
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

















Sensitivity

The sensitivity level (minimum detection limit) of the Emit® II Plus Propoxyphene Assay is 59 ng/mL. This level represents the lowest concentration of propoxyphene that can be distinguished from 0 ng/mL with a confidence level of 95%.

9 REFERENCES

- Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse*. Rockville, MD: National Institute on Drug Abuse (NIDA), NIDA research monograph 73. Department of Health and Human Services; 1986.
- Gilman AG, Rall TW, Nies AS, Taylor P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th ed. New York, NY: Pergamon Press; 1990:510.
- Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man*. 3rd ed. Chicago, IL: Year Book Medical Publishers Inc; 1989.
- Henry JB. *Clinical Diagnosis and Management by Laboratory Methods*. Philadelphia, PA: WB Saunders Co; 1991:866.

Symbols Key

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250 S. Kraemer Blvd.
Brea, CA 92821



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