TECHNICAL BULLETIN

LZI Ketamine Enzyme Immunoassay on Beckman Coulter AU680

For Beckman Coulter, Inc.

REF C68802 (100/37.5 mL R_1/R_2 Kit)

Lin-Zhi International, Inc.

For Sales Outside USA (OUS) Only

Intended Use

The LZI Ketamine Enzyme Immunoassay for Beckman Coulter, Inc. is intended for the qualitative and semi-quantitative determination of norketamine in human urine at the cutoff value of 50 ng/mL when calibrated against norketamine. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. The semi-quantitative mode is for purposes of enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS or LC/MS, or permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical result. A more specific alternative chemical confirmatory method (e.g., gas or liquid chromatography and mass spectrometry) must be used to obtain a confirmed analytical result (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is a preliminary positive.

Calibrators and Controls*

**Calibrators and Controls are sold separately or as a semi-quantitative set and contain negative human urine with sodium azide as a preservative.*

Qualitative Calibration	REF
LZI Norketamine Qualitative Calibrator NKET Cutoff Calibrator (50 ng/mL)	C68804

Semi-Quantitative Calibration	REF
LZI Universal Negative Calibrator	C68807
LZI Norketamine Semi-Quantitative Calibrator Set NKET Low Calibrator (25 ng/mL) NKET Cutoff Calibrator (50 ng/mL) NKET Intermediate #1 Calibrator (100 ng/mL) NKET Intermediate #2 Calibrator (250 ng/mL) NKET High Calibrator (500 ng/mL)	C68803

Controls	REF
LZI Norketamine Level 1 Control NKET Level 1 Control (37.5 ng/mL)	C68805
LZI Norketamine Level 2 Control NKET Level 2 Control (62.5 ng/mL)	C68806

Others

Wedge	REF
OSR Bottle kit, 20 x 60 mL	63093
OSR Bottle kit, 20 x 30 mL	63094

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this technical bulletin have been validated on the Beckman Coulter AU680 automated clinical analyzer.

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay.

For qualitative analysis, use the 50 ng/mL as the cutoff calibrator. The cutoff is normalized to 100. Positive samples are ≥ 100 and are flagged with a (P).

For semi-quantitative analysis, use all six calibrators including the universal negative calibrator. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are available for monitoring of each cutoff level. Use the 37.5 ng/mL and 62.5 ng/mL controls for the 50 ng/mL cutoff level.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact your local Beckman Coulter Representative for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU680 automated chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from six calibrators. Typical results (ng/mL) are as follows:

50 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Norketamine Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
12.5 ng/mL	25 %	22	22 Neg	88	88 Neg
25 ng/mL	50 %	22	22 Neg	88	88 Neg
37.5 ng/mL	75 %	22	22 Neg	88	88 Neg
50 ng/mL	100 %	22	0 Neg/ 22 Pos	88	5 Neg/ 83 Pos
62.5 ng/mL	125 %	22	22 Pos	88	88 Pos
75 ng/mL	150 %	22	22 Pos	88	88 Pos
87.5 ng/mL	175 %	22	22 Pos	88	88 Pos
100 ng/mL	200 %	22	22 Pos	88	88 Pos

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

50 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Norketamine Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
12.5 ng/mL	25 %	22	22 Neg	88	88 Neg
25 ng/mL	50 %	22	22 Neg	88	88 Neg
37.5 ng/mL	75 %	22	22 Neg	88	88 Neg
50 ng/mL	100 %	22	0 Neg/ 22 Pos	88	2 Neg/ 86 Pos
62.5 ng/mL	125 %	22	22 Pos	88	88 Pos
75 ng/mL	150 %	22	22 Pos	88	88 Pos
87.5 ng/mL	175 %	22	22 Pos	88	88 Pos
100 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy: One hundred eleven (111) unaltered clinical urine specimens and pooled urine samples spiked with norketamine were tested with the LZI Ketamine Enzyme Immunoassay and confirmed with LC/MS. Specimens with a combined norketamine and ketamine concentration greater than or equal to 50 ng/mL by LC/MS are defined as positive, and specimens with a combined norketamine and ketamine concentration below 50 ng/mL by LC/MS are defined as negative in the table below. Near cutoff samples are defined as ± 50 % of the cutoff value. The correlation results are summarized as follows:

Semi-Quantitative Accuracy Study:

50 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	2*	2**	6	62	100.0 %
Negative	20	4	15	0	0	90.7 %

The following table summarizes the results for the semi-quantitative discordant samples:

Sample #	NKET LC/MS (ng/mL)	KET LC/MS (ng/mL)	Total NKET + KET LC/MS (ng/mL)	Pos/ Neg Result	AU680 EIA Semi-Quantitative Result (ng/mL)	Pos/ Neg Result
24*	17	0.0	17.0	-	234.2	+
26*	19.6	0.0	19.6	-	235.8	+
31**	14.3	12.8	27.1	-	132.2	+
34**	0.0	32.3	32.3	-	59.1	+

Qualitative Accuracy Study:

50 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	2*	2**	6	62	100.0 %
Negative	20	4	15	0	0	90.7 %

The following table summarizes the results for the qualitative discordant samples:

Sample #	NKET LC/MS (ng/mL)	KET LC/MS (ng/mL)	Total NKET + KET LC/MS (ng/mL)	Pos/ Neg Result	AU680 EIA Qualitative Result (mAU)	Pos/ Neg Result
24*	17	0.0	17.0	-	348.0	+
26*	19.6	0.0	19.6	-	342.7	+
31**	14.3	12.8	27.1	-	214.5	+
34**	0.0	32.3	32.3	-	101.3	+

Calibration Cutoff Average = 77.7 mAU

** Discordant between negative and <50 % cutoff concentration (0.1 – 24.9 ng/mL)*

*** Discordant between 50 % of cutoff and cutoff concentration (25 – 49.9 ng/mL)*

Analytical Recovery: To demonstrate recovery for purposes of sample dilution and quality control of the entire assay range, a drug-free urine pool spiked with norketamine at 500 ng/mL was serially diluted. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value.

Target Concentration (ng/mL)	Determined Concentration Range (ng/mL)	Determined Concentration Average (ng/mL)	Average % Recovery
500	461.7 – 496.6	485.3	97.1 %
450	431.0 – 467.9	453.4	100.7 %
400	374.8 – 418.4	401.0	100.2 %
350	320.6 – 362.7	344.2	98.3 %
300	269.6 – 303.4	287.4	95.8 %
250	239.3 – 247.6	242.7	97.1 %
200	189.6 – 199.7	196.4	98.2 %
150	147.4 – 159.2	153.9	102.6 %
100	85.9 – 91.8	88.9	88.9 %
50	48.9 – 54.5	51.6	103.2 %
25	23.3 – 29.2	26.4	105.4 %
7.5	8.8 – 16.2	12.2	162.3 %
0	-1.1 – 5.6	3.3	N/A

Specificity: Test compounds were spiked into a drug-free urine pool to various concentrations and evaluated with the assay's calibration curve in both qualitative and semi-quantitative modes.

The following table lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative). Compounds tested at high concentration (100000 ng/mL) with results below the cutoff value were listed as Not Detected (ND).

Ketamine and Metabolites:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Norketamine	50	100.00 %
Ketamine	55	90.91 %
Dehydronorketamine	2000	2.50 %
Hydronorketamine	100000	ND

Structurally Related Compounds:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Deschloroketamine	1600	3.13 %
Methoxetamine	100000	0.05 %
Phencyclidine	100000	0.05 %

Additional Information

For more detailed information on AU 8 series and DxC AU Systems, refer to the appropriate system manual.

Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

Bibliography

1. Urine Testing for Drug 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, **23**(82):7920-7970 (2017).

A point (period/stop) is always used in this instruction for use document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Additions, deletions, or changes are indicated by a change bar in the margin.

For instructions for use (including translations) please visit: https://www.lin-zhi.com/bci_applications/

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