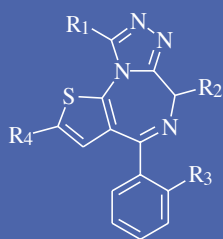


General Structure of  
"Classic" Benzodiazepine



General Structure of  
Thienotriazolodiazepine

## ARK™ HS Benzodiazepine II Assay

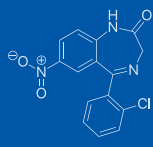
The ARK HS Benzodiazepine II Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of benzodiazepines in human urine at cutoff concentrations of 100 ng/mL and 200 ng/mL. The assay provides a simple and rapid analytical screening procedure for detecting benzodiazepines in urine and is designated for professional use on automated clinical chemistry analyzers.



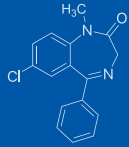
### KEY POINTS

- Convenient, liquid-stable, ready-to-use homogeneous enzyme immunoassay
- Qualitative and/or semi-quantitative applications available for clinical chemistry systems
- 0 – 3,000 ng/mL calibration range; 100 ng/mL and 200 ng/mL cutoffs
- Excellent sensitivity and specificity for detection of benzodiazepines in human urine

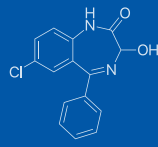
*Next Generation Assays*



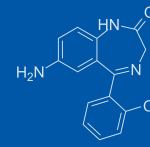
Clonazepam



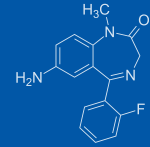
Diazepam



Oxazepam



7-Aminoclonazepam



7-Aminoflunitrazepam

“Classic” Benzodiazepines

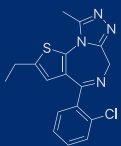
“7-Amino” metabolites of Benzodiazepines

The ARK HS Benzodiazepine II Assay detects classic and designer benzodiazepines plus the 7-amino and glucuronide metabolites of benzodiazepines with no sample pretreatment required. Minimize false negative (FN) benzodiazepines screening assay results.

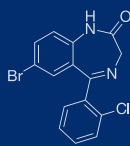


“Designer” Benzodiazepines

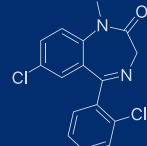
“Glucuronide” metabolites of Benzodiazepines



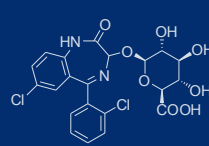
Etizolam



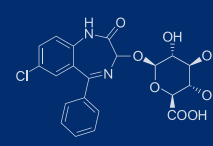
Phenazepam



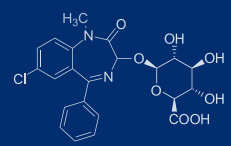
Diclazepam



Lorazepam Glucuronide

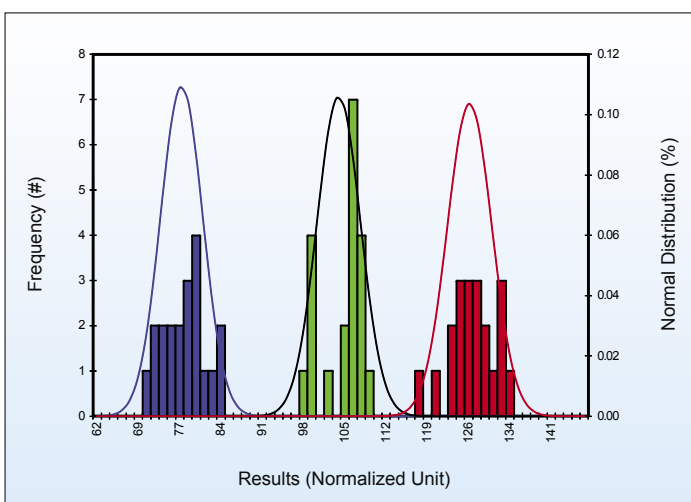


Oxazepam Glucuronide

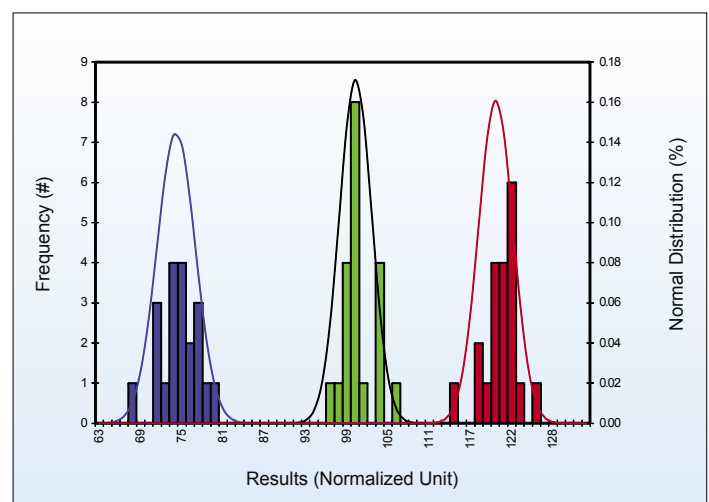


Temazepam Glucuronide

QUALITATIVE PRECISION



Qualitative Control Precision vs 100 ng/mL Cutoff Calibrator



Qualitative Control Precision vs 200 ng/mL Cutoff Calibrator

## ACCURACY – ANALYTICAL RECOVERY

Theoretical Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
25.0	23.8	95.1
50.0	54.8	109.5
100.0	99.6	99.6
250.0	242.2	96.9
500.0	481.4	96.3
1000.0	951.9	95.2
1500.0	1422.4	94.8
2000.0	1929.9	96.5
2500.0	2333.7	93.3
3000.0	3107.7	103.6

## SEMI-QUANTITATIVE PRECISION

### 100 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	1.1	160 Negative
25.0	-75	160	23.2	160 Negative
50.0	-50	160	50.5	160 Negative
75.0	-25	160	74.0	160 Negative
100.0	Cutoff	160	98.6	93 Negative/67 Positive
125.0	+25	160	123.3	160 Positive
150.0	+50	160	145.2	160 Positive
175.0	+75	160	170.5	160 Positive
200.0	+100	160	187.6	160 Positive

### 200 ng/mL Cutoff

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	1.1	160 Negative
50.0	-75	160	50.5	160 Negative
100.0	-50	160	98.6	160 Negative
150.0	-25	160	145.2	160 Negative
200.0	Cutoff	160	187.6	147 Negative/13 Positive
250.0	+25	160	240.3	160 Positive
300.0	+50	160	293.2	160 Positive
350.0	+75	160	342.8	160 Positive
400.0	+100	160	391.1	160 Positive

Pooled Urine Samples containing etizolam were assayed in quadruplicate twice a day for 20 days. CLSI Guideline EP5-A3.

## ANALYTICAL SPECIFICITY

### Structurally Related Compounds

Compounds	Compound conc. (ng/mL) approximately equivalent to 100 ng/mL Cutoff	Compound conc. (ng/mL) approximately equivalent to 200 ng/mL Cutoff
α-OH-alprazolam	55	100
α-OH-midazolam	11	16
α-OH-Triazolam	20	35
2-OH-ethylflurazepam	70	125
3-OH-flubromazepam	19	27
3-OH Phenazepam	15	22
4-OH-alprazolam	110	190
7-aminoclonazepam	40	75
7-aminoflunitrazepam	150	270

Compounds	Compound conc. (ng/mL) approximately equivalent to 100 ng/mL Cutoff	Compound conc. (ng/mL) approximately equivalent to 200 ng/mL Cutoff
7-aminonimetazepam	600	1200
7-aminonitrazepam	400	800
Alprazolam	80	130
Bromazepam	140	270
Chlordiazepoxide	55	100
Clobazam	180	400
Clonazepam	100	180
Delorazepam	10	15
Demoxepam	100	180
Desalkylflurazepam	15	25
Clonazolam	350	800
Clorazepate	45	70
Deschloroetizolam	450	900
Diazepam	65	100
Diclazepam	15	25
Estazolam	60	85
Flualprazolam	28	45
Flubromazepam	25	38
Flubromazolam	40	68
Flunitrazepam	450	850
Flurazepam	350	650
Halazepam	2,500	5,000
Ketazolam	2,200	4,000
Loprazolam	130	260
Lorazepam	12	16.5
Lorazepam glucuronide	13	20
Lormetazepam	17	23
Meclonazepam	36	57
Medazepam	250	500
Midazolam	16	25
N-Desmethylclobazam	160	320
N-desmethylflunitrazepam	150	300
Nimetazepam	1,350	3,000
Nitrazepam	500	950
Norchlorodiazepoxide	75	120
Nordiazepam	43	57
Oxazepam	38	55
Oxazepam Glucuronide	20	30
Phenazepam	15	20
Prazepam	1,700	3,400
Pyrazolam	350	700
Temazepam	85	130
Temazepam glucuronide	35	70
Tetrazepam	600	1,200
Triazolam	27	45

## METHOD COMPARISON

A total of one hundred sixty-three (163) unaltered, un-pretreated with glucuronidase, clinical human urine specimens that are not individually identifiable were analyzed for benzodiazepine at the two cutoff levels with the ARK HS Benzodiazepine II Assay in semiquantitative modes and the results were compared to LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory. Briefly, the method involves treating specimens with glucuronidase, adding internal standards, and injecting into a column for LC-MS/MS. Detection peaks and their limits of quantitation (LoQ) in ng/mL are: 7-aminoclonazepam (5); alprazolam (1); hydroxylalprazolam (1); lorazepam (10); diazepam (5); nordiazepam (5); oxazepam (5); temazepam (1);

midazolam (1); hydroxymidazolam (1). Results are summarized in the tables below, where the LC-MS/MS result represents the sum across all benzodiazepine peaks identified.

All ARK-positive samples in this study were confirmed by LC-MS/MS to have benzodiazepine concentrations of at least 20 ng/mL.

#### 100 ng/mL Cutoff

ARK Result	LC-MS/MS Result (ng/mL)			
	Less than 50% of cutoff (< 50 ng/mL)	Between 50% below the Cutoff and the Cutoff (50 – 99.9 ng/mL)	Between the Cutoff and 50% above the Cutoff (100 – 149.9 ng/mL)	Equal or Greater than 50% above the Cutoff (≥ 150 ng/mL)
Negative (< 100 ng/mL)	71	3	2	1*
Positive (≥ 100 ng/mL)	8**	9	9	60

Sample ID	ARK Assay (ng/mL)	LC-MS/MS (ng/mL)	Benzodiazepines present by LC-MS/MS
62*	96.3	216.9	Alprazolam; hydroxyalprazolam
8**	118.2	39.1	7-aminoclonazepam
30**	661.8	26.4	Lorazepam
41**	183.8	23.9	Nordiazepam, oxazepam, temazepam
42**	466.3	27.1	Nordiazepam, oxazepam, temazepam
43**	520.1	23.5	Nordiazepam, oxazepam, temazepam
90**	> 3,000	46.0	Lorazepam
91**	192.3	28.4	Lorazepam
93**	340.6	29.6	Lorazepam

\*\*Strong reactivity to 7-aminoclonazepam, diazepam, lorazepam-glucuronide, oxazepamglucuronide and temazepam-glucuronide contributed to the positive results obtained with the ARK HS Benzodiazepine II Assay.

#### 200 ng/mL Cutoff

ARK Result	LC-MS/MS Result (ng/mL)			
	Less than 50% of cutoff (< 100 ng/mL)	Between 50% below the Cutoff and the Cutoff (100 – 199.9 ng/mL)	Between the Cutoff and 50% above the Cutoff (200 – 299.9 ng/mL)	Equal or Greater than 50% above the Cutoff (≥ 300 ng/mL)
Negative (< 200 ng/mL)	78	6	2	2†
Positive (≥ 200 ng/mL)	13††	11	11	40

Sample ID	ARK Assay (ng/mL)	LC-MS/MS (ng/mL)	Benzodiazepines present by LC-MS/MS
55†	180.9	386.1	Alprazolam; hydroxyalprazolam
61†	176.6	543.4	Alprazolam; hydroxyalprazolam
30††	661.8	26.5	Lorazepam
35††	1,620.1	90.3	Lorazepam
42††	466.3	27.1	Diazepam, nordiazepam, oxazepam, temazepam
43††	520.1	23.5	Nordiazepam, oxazepam, temazepam
47††	1,291.9	66.3	Nordiazepam, oxazepam, temazepam
48††	838.8	66.5	Nordiazepam, oxazepam, temazepam
72††	1,117.9	75.4	Nordiazepam, oxazepam, temazepam
82††	811.7	73.0	Nordiazepam, oxazepam, temazepam
90††	> 3,000	46.0	Lorazepam
93††	340.6	29.6	Lorazepam
94††	1,124.9	60.6	Lorazepam
96††	697.6	57.5	Lorazepam
97††	815.2	68.0	Lorazepam

††Strong reactivity with diazepam, lorazepam-glucuronide, oxazepam-glucuronide and temazepam-glucuronide contributed to the positive results obtained with the ARK HS Benzodiazepine II Assay.

## SAFETY AND STABILITY

### Reagent on-board stability

Up to at least 60 days

### Shelf Life of Reagents, Calibrators, and Controls

18 months from date of manufacturing

### Safety

Nonhazardous preservatives

Contains sodium azide ≤ 0.09%

Results shown are typical and may vary among laboratory analyzers.

## ORDERING INFORMATION

ARK™ HS Benzodiazepine II Assay	5073-0001-00	R1 28mL, R2 14mL
	5073-0001-01	R1 115mL, R2 58mL
	5073-0001-02	R1 500mL, R2 250mL
ARK™ HS Benzodiazepine II Calibrator	5073-0002-00	5 x 10mL
	5073-0002-01	2 x 10mL; Negative
	5073-0002-02	2 x 10mL; 100 ng/mL Cutoff
	5073-0002-03	2 x 10mL; 200 ng/mL Cutoff
ARK™ HS Benzodiazepine II Control	5073-0003-00	2 x 10mL; LOW 75 ng/mL 2 x 10mL; HIGH 125 ng/mL
	5073-0003-01	2 x 10mL; LOW 150 ng/mL 2 x 10mL; HIGH 250 ng/mL

### ARK Diagnostics, Inc.

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### For Customer Support:

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