ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ *Pregabalin Urine Assay*

This ARK Diagnostics, Inc. package insert for the ARK Pregabalin Urine Assay must be read prior to use. Package insert instructions must be followed accordingly. The assay provides a simple and rapid analytical screening procedure for detecting pregabalin in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.
48089 Fremont Blvd
Fremont, CA 94538 USA
Tel: 1-877-869-2320
Fax: 1-510-270-6298
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 / R2	Reagent 1/ Reagent 2
Rx Only	For Prescription Use Only		

1 NAME

ARK™ *Pregabalin Urine Assay*

2 INTENDED USE

The ARK Pregabalin Urine Assay is intended for the qualitative and/or semiquantitative determination of pregabalin in human urine at a cutoff concentration of 500 ng/mL. The assay provides a simple and rapid analytical screening procedure for detecting pregabalin in urine and is designated for professional use on automated clinical chemistry analyzers.

The ARK Pregabalin Urine 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) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) are the preferred confirmatory methods.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

3 SUMMARY AND EXPLANATION OF THE TEST

In Europe, pregabalin is approved for the treatment of epilepsy (partial seizures), neuropathic pain and generalized anxiety disorder.[2] In the United States, pregabalin is approved for the treatment of epilepsy (partial seizures), neuropathic pain associated with diabetes, postherpetic neuropathy, and fibromyalgia.[3] Pregabalin is not metabolized in the body to a significant degree but is almost exclusively excreted unchanged in the urine by glomerular filtration.[4]

Pregabalin is classified as a schedule V drug in the U.S. Drug Enforcement Administration's Controlled Substances Act.[5] In the European Union, pregabalin is not a controlled substance subjected to special or restricted prescription, but a warning related to its abuse potential[6-8] was added to the Summary of Product Characteristics in June 2010.[9]

The ARK Pregabalin Urine Assay tests for pregabalin in human urine and gives a positive result if this drug is present at concentrations equal to or greater than the cutoff.

4 PRINCIPLES OF THE PROCEDURE

The ARK Pregabalin Assay is a homogeneous enzyme immunoassay technique used for the analysis of a specific compound in human urine. The assay is based on competition between drug in the specimen and drug labeled with the recombinant glucose-6-phosphate dehydrogenase (rG6PDH) 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 in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENTS

REF	Product Description	Quantity/Volume
5035-0001-00	**ARK Pregabalin Urine Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to pregabalin, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme pregabalin derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), buffer, sodium azide and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Pregabalin Urine Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK Pregabalin Urine products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

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6 WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Do not use reagents after the expiration date.
- Reagents contain ≤0.09% sodium azide.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human urine is required. Treat as potentially infectious material.
- Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical and physical integrity of the urine sample from the time it is collected until the time it is assayed, including during transport. Fresh urine specimens are suggested.
- Cap the urine sample immediately after collection, store at 2-8°C (36–46°F) and assay within 7 days after collection. If the assay can't be performed within 7 days, store the urine sample frozen.
- To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- The recommended pH range for urine specimens is 4.0 – 10.0.
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

8 PROCEDURE

Materials Provided

ARK Pregabalin Urine Assay – REF 5035-0001-00

Materials Required – Provided Separately

ARK Pregabalin Urine Calibrator – REF 5035-0002-00
Quality Controls – ARK Pregabalin Urine Control – REF 5035-0003-00

Instruments

Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2 Refer to the instrument-specific operator's manual for daily maintenance. Consult the analyzer-specific application sheet for programming the pregabalin assay or contact Customer Support.

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual.

Qualitative Results

Use the 500 ng/mL Calibrator C as a Cutoff Calibrator to distinguish negative and positive samples. Run the Low and High Controls as Negative and Positive respectively. All qualitative testing results are expressed as enzymatic rate (mA/min). Report test results less than the rate for the Cutoff Calibrator as Negative. Report results equal to or greater than the rate for the Cutoff Calibrator as Positive.

Semiquantitative Results

To estimate the concentration of pregabalin, perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with ARK Low and High quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC)

Laboratories should establish QC procedures for the ARK Pregabalin Urine Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Each laboratory should establish its own ranges for each new lot of controls. The ARK Pregabalin Urine Control is a non-assayed control intended for quality control of the ARK Pregabalin Urine Assay when run in either the qualitative or semiquantitative mode.

In Qualitative Mode the Low Control should be Negative and the High Control should be Positive relative to the 500 ng/mL Cutoff Calibrator.

9 RESULTS

Qualitative Analysis - Negative Results

A specimen that gives a rate value less than the ARK Pregabalin Urine Calibrator cutoff rate value is interpreted as negative; either the specimen does not contain pregabalin or pregabalin is present in a concentration below the cutoff level of this assay.

Qualitative Analysis - Positive Results

A specimen that gives a rate value equal to or greater than the ARK Pregabalin Urine Calibrator cutoff rate value is interpreted as positive, indicating that pregabalin is present.

Semiquantitative Analysis

Calibrating the ARK Pregabalin Urine Assay in the semiquantitative mode makes it possible to estimate the concentration of pregabalin. The semiquantitative measurement range of the ARK Pregabalin Urine Assay is 200 to 2000 ng/mL based on linearity and recovery.

The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for confirmation by the analytical method being used. Semiquantitation also permits the laboratory to establish quality control procedures and assess control performance. Refer to the Analytical Recovery section.

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

10 LIMITATIONS

- The assay is designated for use with human urine only.
- ARK pregabalin assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Pregabalin Urine Assay indicates only the presence of pregabalin and does not necessarily correlate with the extent of physiological and psychological effects.
- Boric acid is not recommended as a preservative.
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- It is possible that substances other than those investigated in the specificity study may interfere with the test and cause false results.
- To maintain sample stability store processed patient samples frozen at -20 °C.

11 EXPECTED VALUES

Qualitative assay results distinguish between positive and negative specimens; positive indicating that specimens contain pregabalin at concentrations equal to or greater than 500 ng/mL. The actual concentration cannot be determined. A confirmatory method is required.

When used semiquantitatively, the assay estimates the concentration of pregabalin within 200 to 2000 ng/mL. Extremely high concentrations of gabapentin ≥ 4.5 mg/mL may cause a positive result.

12 SPECIFIC PERFORMANCE CHARACTERISTICS

The data appearing in this section were collected on the Beckman Coulter AU480® clinical chemistry analyzer using the ARK Pregabalin Urine Assay.

Method Comparison

Qualitative and Semiquantitative Results

One hundred thirty six samples (136) were analyzed by ARK Pregabalin Assay and by LC-MS/MS. Both methods used a cutoff of 500 ng/mL. Three (3) samples were within ± 50% of the cutoff by LC-MS/MS.

Sixty nine (69) samples showed positive by both methods, while sixty seven (67) samples showed negative results by both methods. Data are summarized in Tables 1 and 2.

Table 1 – Qualitative Accuracy Summary

		LC-MS/MS 500 ng/mL Cutoff	
		(+)	(-)
ARK Pregabalin 500 ng/mL Cutoff	(+)	69	0
	(-)	0	67

Table 2 – Semiquantitative Accuracy Summary

		LC-MS/MS				
		LOW NEG Less than 50% below the cutoff (< 250 ng/mL)	**NEG Within 50% below the cutoff (250-500 ng/mL)**	**POS Within 50% above the cutoff (500-750 ng/mL)**	**HIGH POS Greater than 50% above the cutoff (> 750 ng/mL)**	**Agreement (%)**
Qualitative Summary						
ARK	POS	0	0	0	69	100%
	NEG	64	3	0	0	100%
Semiquantitative Summary						
ARK	POS	0	0	0	69	100%
	NEG	64	3	0	0	100%

Precision

Precision was determined by assaying urine pools spiked with pregabalin for 20 days, 2 runs per day in quadruplicate (N=160). Precision data were calculated according to the Clinical Laboratory Standards Guideline Protocol EP05-A3.

Table 3 – Precision - Qualitative Analysis

Urine Spiked Samples		N=160	Within Run		Total Precision	
(ng/mL)	% of cutoff	Mean (mA/min)	SD	CV (%)	SD	CV (%)
0	-100	426	2.1	0.5	2.6	0.6
250	-50	477	2.6	0.5	3.5	0.7
375	-25	488	3.2	0.6	3.7	0.8
500	cutoff	498	2.1	0.4	2.9	0.6
625	+25	508	2.2	0.4	3.2	0.6
750	+50	513	2.1	0.4	3.3	0.7
1000	+100	523	2.3	0.4	3.0	0.6

Table 4 – Precision - Semiquantitative Analysis

Urine Spiked Samples		N=160	Within Run		Total Precision	
(ng/mL)	% of cutoff	Mean (ng/mL)	SD	CV (%)	SD	CV (%)
0	-100	0.6	-	-	-	-
250	-50	248	20.2	8.2	26.1	10.5
375	-25	371	31.7	8.5	39.0	10.5
500	cutoff	478	27.5	5.8	35.7	7.5
625	+25	600	33.3	5.5	46.8	7.8
750	+50	705	33.7	4.8	52.2	7.4
1000	+100	917	50.9	5.5	69.7	7.6

Analytical Recovery

Accuracy (analytical recovery) was performed by adding concentrated pregabalin drug into human urine negative for pregabalin. A stock concentrate of highly pure pregabalin was added volumetrically to human urine negative for pregabalin, representing drug concentrations across the assay range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer.

Qualitative Results

In qualitative analysis, the ARK Pregabalin Urine Assay correctly identified the mean rate of spiked specimens containing less than the cutoff as negative and the mean rate of spiked specimens containing greater than the cutoff as positive 100% of the time.

Semiquantitative Results

Drug-free urine was spiked with concentrations of pregabalin at levels ranging from 50 to 1500 ng/mL. For each known concentration, drug recovery was calculated using the mean concentration obtained by the ARK Pregabalin Urine Assay. Semiquantitative results are shown in Table 5.

Table 5 – Analytical Recovery of Semiquantitative Results

Expected Pregabalin Concentration (ng/mL)	Mean Pregabalin Concentration ARK Pregabalin Urine Assay (ng/mL)	Recovery (%)
50	41.5	83.0
100	77.6	77.6
250	236.4	94.6
625	585.7	93.7
750	714.1	95.2
1500	1438.7	95.9

Linearity

Linearity was assessed using the semiquantitative mode as suggested in CLSI/NCCLS Protocol EP6-A. A 2500 ng/mL urine sample was prepared and dilutions were made proportionally with drug-free human urine. Pregabalin concentrations ranged from 100 to 2000 ng/mL. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values. Results are shown in Table 6.

Table 6 – Linearity of Semiquantitative Results

Estimated Value (ng/mL)	Results (ng/mL)	Recovery (%)	1st Order Predicted Results	2nd Order Predicted Results	Difference (%)
100	107.5	107.5	88.5	113.2	27.9
200	200.8	100.4	186.9	201.9	8.0
300	299.7	99.9	285.4	291.8	2.3
400	375.1	93.8	383.8	382.9	-0.2
600	574.2	95.7	580.6	568.7	-2.1
800	743.1	92.9	777.5	759.2	-2.3
1000	977.8	97.8	974.3	954.6	-2.0
1200	1162.7	96.9	1171.1	1154.8	-1.4
1600	1520.0	95.0	1564.8	1569.6	0.3
2000	2010.7	100.5	1958.5	2003.5	2.3

Specificity

The ARK Pregabalin Urine Assay detects pregabalin in human urine. Pregabalin undergoes negligible metabolism in humans.[10]

Table7 lists Gabapentin and L-amino acids that produce a negative result at the concentration tested and did not yield a response equivalent to the 500 ng/mL cutoff. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 7 may combine to produce a rate equal to or greater than the cutoff calibrator. Data presented are representative of typical performance of this assay.

Table 7 – Concentrations of Gabapentin and Amino Acids Showing a Negative Response versus the 500 ng/mL Pregabalin Cutoff

Compound	Conc. Tested (µg/mL)	Cross-reactivity (%)
Gabapentin	4000	0.01
L-Arginine	200	0.00
L-Asparagine	200	0.00
L-Aspartic Acid	200	0.00
L-Cysteine	200	0.00
L-Glutamic Acid	200	0.00
L-Glycine	200	0.00
L-Histidine	200	0.00
L-Isoleucine	200	0.00
L-Leucine	200	0.02
L-Methionine	200	0.04
L-Phenylalanine	200	0.00
L-Serine	200	0.00
L-Threonine	200	0.00
L-Tyrosine	200	0.00
L-Alanine	200	0.00
L-Lysine	200	0.00
L-Proline	200	0.00
L-Valine	200	0.00
L-Tryptophan	200	0.00
L-Glutamine	200	0.00

Structurally Unrelated Compounds

The Low Control (250 ng/mL) and the High Control (750 ng/mL) represent pregabalin levels that are ± 50% of the 500 ng/mL Calibrator C. Compounds that are not structurally related to pregabalin were spiked into each of these controls and then tested in both qualitative and semiquantitative modes of analysis.

Qualitative Results

In the qualitative analysis, the ARK Pregabalin Urine Assay correctly identified the mean rate of the control at -50% of the cutoff as negative 100% of the time and the mean rate of the control at +50% of the cutoff as positive 100% of the time.

Semiquantitative Results

In the semiquantitative analysis, the two levels of controls at ± 50% of the cutoff concentration did not yield a false response relative to the cutoff. Results are shown in Table 8.

Table 8 – Structurally Unrelated Compounds: Semiquantitative Results

Compound	Conc. Tested (µg/mL)	Sample (-50% Control) (%) Cross-reactivity	Sample (+50% Control) (%) Cross-reactivity
6-Acetyl morphine	10	-0.11	-0.27
Amitriptyline	100	0.00	0.09
Amoxicillin	100	0.04	0.03
Amphetamine	100	0.21	0.46
Benzoylecgonine	100	0.00	-0.14

Table 8 – Structurally Unrelated Compounds: Semiquantitative Results

Compound	Conc. Tested (µg/mL)	Sample (-50% Control) (%) Cross-reactivity	Sample (+50% Control) (%) Cross-reactivity
Carbamazepine	100	0.13	0.19
Chlorpromazine	100	0.04	0.03
Clomipramine	100	0.01	-0.03
Cimetidine	500	0.00	0.00
Codeine	100	-0.02	-0.01
Desipramine	100	-0.01	-0.05
Dextromethorphan	200/150	-0.01	-0.05
Dihydrocodeine	100	-0.03	-0.11
Doxepine	200	-0.01	-0.07
Ephedrine	200/150	-0.03	-0.07
Fentanyl	100	-0.04	-0.10
Fluoxetine	100	-0.02	-0.10
Fluphenazine	100	0.03	-0.12
Heroin	100	-0.06	-0.20
Hydrocodone	200	-0.03	-0.09
Hydromorphone	200	-0.02	-0.08
Imipramine	100	-0.03	-0.03
Levorphanol	50	-0.01	-0.07
Meperidine	100	-0.03	-0.08
Meprotiline	100	-0.01	-0.09
Methadone	100	-0.03	-0.08
Metronidazole	300	-0.01	0.01
Morphine	100	-0.03	-0.13
Morphine-3-glucuronide	50	-0.03	-0.20
Nalbuphine	100	-0.02	-0.02
Naltrexone	50	-0.05	-0.15
Norcodeine	50	0.01	-0.11
Normorphine	50	-0.03	-0.15
Nortriptyline	50	0.11	-0.07
Oxazepam	100	-0.02	-0.11
Oxycodone	100	-0.04	-0.13
Pentazocine	50	-0.03	-0.17
Phencyclidine	50	0.13	-0.17
Phenobarbital	100	-0.03	-0.14
Ranitidine	100	0.02	-0.06
Secobarbital	100	-0.04	-0.05
Thioridazine	100	-0.03	-0.03
Tramadol	100	-0.02	-0.06

Non-Interfering Substances

The substances were spiked into two levels of controls at ± 50% of the cutoff concentration. The results for both qualitative and semiquantitative mode are presented below.

Qualitative Results

In the qualitative analysis, the ARK Pregabalin Urine Assay correctly identified the mean rate of the control at -50% of the cutoff as negative 100% of the time and the mean rate of the control at +50% of the cutoff as positive 100 % of the time.

Semiquantitative Results

In the semiquantitative analysis, the two levels of controls at ± 50% of the cutoff concentration did not yield a false response relative to the cutoff. Results are shown in Table 9.

Table 9 — Interfering Substances

Interfering Substance	Level Tested (mg/dL)	Sample Mean (-50% Control) (ng/mL)	Sample Mean (+50% Control) (ng/mL)
Acetaminophen	10	231.1	648.4
Acetone	1000	261.6	698.6
Acetylsalicylic Acid	10	260.0	642.0
Ascorbic Acid	200	245.7	688.1
Caffeine	10	250.4	704.0
Creatinine	400	272.4	683.5
Ethanol	10	232.9	687.1
Galactose	10	226.7	681.6
Glucose	3000	231.0	663.3
Hemoglobin	300	188.6	536.6
Human Serum Albumin	500	259.8	626.6
Ibuprofen	10	235.5	655.4
Oxalic Acid	30	228.1	674.3
Riboflavin	3.75	236.0	648.9
Sodium Chloride	900	244.9	680.0
Urea	1000	237.9	738.4

Specific Gravity and p

Urine samples with specific gravity values from 1.003 to 1.035 g/mL and pH values ranging from 4.0 to 10.0 were tested in the presence of 250 and 750 ng/mL of pregabalin. No interference was observed.

13 REFERENCES

1. Hawks RL. 1986. Analytical methodology. In Hawks RL, Chiang CN, eds. Urine testing for drugs of abuse. NIDA Research Monograph. **73**:30-41.
2. European Medicines Agency. Lyrica–summary of product characteristics. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000546/WC500046602.pdf. Accessed July 2, 2012.
3. U.S. Food and Drug Administration. Label approved on August 24, 2011, for Lyrica. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022488-s004-0211446s025lbl.pdf. Accessed July 2, 2012.
4. Bockbrader HN, et al. 2010. A comparison of the pharmacokinetics and pharmacodynamics of pregabalin and gabapentin. Clin Pharmacokinet. **49**:661–669.
5. Drug Enforcement Administration, Department of Justice. 2005. Schedules of controlled substances: placement of pregabalin into schedule V. Final rule. Fed Regist. **70**:43633–43635.
6. Grosshans M, et al. 2013. Pregabalin abuse among opiate addicted patients. Eur J Clin Pharmacol. **69**:2021-2025.
7. Schifano F. 2014. Misuse and abuse of pregabalin and gabapentin: Cause for concern? In: CNS Drugs, Springer International Publishing, Switzerland.
8. Baird C, et al. 2014. Gabapentinoid abuse in order to potentiate the effect of methadone: A survey among substance misusers. European Addiction Research **20**: 115-118.
9. European Medicines Agency (EMA). Lyrica. Procedural steps taken after authorisation. Available at: http://www.ema.europa.eu/docs/en GB/document_library/EPAR - Procedural steps taken and scientific information after authorisation/ human/ 000546/WC500046604.pdf. Accessed July 2, 2012.
10. McElroy SL, Keck PE, Post RM, eds. 2008. Antiepileptic Drugs to Treat Psychiatric Disorders. INFRMA-HC. p. 370.

14 TRADEMARKS

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Fremont, CA 94538 USA

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