ARK Diagnostics, Inc.

For Export Only – Not For Sale in USA

ARK™ *Pregabalin II Assay*

This ARK Diagnostics, Inc. package insert for the ARK Pregabalin II 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.

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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
	Consult Instructions for Use	R1 / R2	Reagent 1/ Reagent 2
	Temperature limitation	IVD	*In Vitro* Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 NAME

ARK™ *Pregabalin II Assay*

2 INTENDED USE

The ARK Pregabalin II Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of pregabalin in human urine at a cutoff concentration of 500 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This *in vitro* diagnostic device is for prescription use only.

The semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK Pregabalin II Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed positive analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

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 subjected to special or restricted prescription and a warning related to its abuse potential[6-8] was added to the Summary of Product Characteristics in June 2010.[9]

The ARK Pregabalin II 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 II Assay is a homogeneous enzyme immunoassay method used for the analysis of pregabalin in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration. 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 G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENTS

REF	Product Description	QTY/ VOL
5059-0001-00	**ARK Pregabalin II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal 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

REF	Product Description	QTY/ VOL
5059-0001-01	**ARK Pregabalin II Assay** Reagent R1 – Antibody/Substrate Rabbit monoclonal antibodies to pregabalin, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme Pregabalin derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), buffer, sodium azide and stabilizers	1 X 58 mL

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Reagent Handling and Storage

ARK Pregabalin II 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 II 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.

6 WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use. For prescription use only.
- 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 refrigerated at 2-8°C (36–46°F) and assay within 7 days after collection. If the assay cannot be performed within 7 days, store the urine sample frozen at -20°C.[10,11]
- 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 – 11.0.[12]
- 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 II Assay – REF 5059-0001-00 or 5059-0001-01

Materials Required – Provided Separately

ARK Pregabalin II Calibrator – REF 5059-0002-00
Quality Controls – ARK Pregabalin II Control – REF 5059-0003-00

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 ARK Pregabalin II 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 ARK Pregabalin II Low (250 ng/mL) and High (750 ng/mL) Controls as Negative and Positive respectively. Report test results less than the response value for the Cutoff Calibrator as Negative. Report test results equal to or greater than the response value for the Cutoff Calibrator as Positive.

Semiquantitative Results

Perform a 5-point calibration procedure; test calibrators in duplicate. Verify the calibration curve with the ARK Pregabalin II Low (250 ng/mL) and High (750 ng/mL) quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK Pregabalin II calibrator level (2000 ng/mL) may be diluted in ARK Pregabalin II Calibrator A (Negative urine) and retested.

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
- A stored calibration curve was effective up to at least 6 days based on supporting data

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Pregabalin II 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. Control results should fall within established ranges as determined by laboratory procedures and guidelines. The ARK Pregabalin II Control is intended for use in quality control of the ARK Pregabalin II Assay.

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 AND EXPECTED VALUES

The actual pregabalin concentration cannot be determined. A confirmatory method is required.

Qualitative Analysis - Negative Results

A specimen that gives a response value less than the ARK Pregabalin II Calibrator C Cutoff response 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 response value equal to or greater than the ARK Pregabalin II Calibrator C Cutoff response value is interpreted as positive, indicating that pregabalin is present.

Semiquantitative Analysis

Semiquantitative results for positive specimens enable the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semiquantitative results also permit the laboratory to establish quality control procedures and assess reproducibility. Specimens with sample results above the highest ARK Pregabalin II calibrator level (2000 ng/mL) may be diluted in ARK Pregabalin II Calibrator A (Negative urine) and retested.

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 II Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Pregabalin II Assay indicates only the presence of pregabalin and does not necessarily correlate with the extent of physiological and psychological effects.
- 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.

11 SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were collected on the Beckman Coulter AU680® automated clinical chemistry analyzer using the ARK Pregabalin II Assay.

Precision

Drug-free, negative human urine was supplemented with pregabalin (0.0 to 1000.0 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated both qualitatively and semiquantitatively. Results are summarized in the tables below.

Qualitative Precision

Human Urine (ng/mL)	% Cutoff	# of Determinations	Qualitative Precision Results
0.0	-100	160	160 Negative
125.0	-75	160	160 Negative
250.0	-50	160	160 Negative
375.0	-25	160	160 Negative
500.0	Cutoff	160	18 Negative/142 Positive
625.0	+25	160	160 Positive
750.0	+50	160	160 Positive
875.0	+75	160	160 Positive
1000.0	+100	160	160 Positive

Semiquantitative Precision

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Semiquantitative Precision Results
0.0	-100	160	2.6	160 Negative
125.0	-75	160	133.6	160 Negative
250.0	-50	160	263.3	160 Negative
375.0	-25	160	392.3	160 Negative
500.0	Cutoff	160	525.3	19 Negative/141 Positive
625.0	+25	160	645.8	160 Positive
750.0	+50	160	786.6	160 Positive
875.0	+75	160	882.6	160 Positive
1000.0	+100	160	1048.3	160 Positive

Analytical Recovery

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with 2400.0 ng/mL of pregabalin and dilutions were made proportionally with drug-free human urine. Pregabalin concentrations ranged from 50.0 to 2000.0 ng/mL. At each level, percentage recovery was calculated based on the mean concentration (N=6) compared to the expected concentration. Results are summarized in the table below.

Theoretical Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
50.0	56.6	113.2
100.0	110.1	110.1
200.0	195.0	97.5
300.0	313.1	104.4
400.0	412.3	103.1
600.0	628.5	104.8
800.0	845.9	105.7
1000.0	1054.3	105.4
1200.0	1274.3	106.2
1600.0	1665.2	104.1
2000.0	2127.7	106.4

Analytical Specificity

Gabapentin and Amino Acids

The ARK Pregabalin II Assay detects pregabalin in human urine. Pregabalin undergoes negligible metabolism in humans.[13]

All compounds tested were added to drug-free, negative human urine and tested with the ARK Pregabalin II Assay in both qualitative and semiquantitative modes.

The table below 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 this table may combine to produce a rate equal to or greater than the cutoff calibrator. Data presented are representative of typical performance of this assay.

Compound	Concentration Tested (ng/mL)	Semiquantitative Mode Result (Positive/Negative)	Qualitative Mode Result (Positive/Negative)
Gabapentin	5,000,000	Negative	Negative
L-Alanine	200,000	Negative	Negative
L-Arginine	200,000	Negative	Negative
L-Asparagine	200,000	Negative	Negative
L-Aspartic Acid	200,000	Negative	Negative
L-Cysteine	200,000	Negative	Negative
L-Glutamic Acid	200,000	Negative	Negative
L-Glutamine	200,000	Negative	Negative
L-Glycine	200,000	Negative	Negative
L-Histidine	200,000	Negative	Negative
L-Isoleucine	200,000	Negative	Negative
L-Leucine	200,000	Negative	Negative
L-Lysine	200,000	Negative	Negative
L-Methionine	200,000	Negative	Negative
L-Phenylalanine	200,000	Negative	Negative
L-Proline	200,000	Negative	Negative
L-Serine	200,000	Negative	Negative
L-Threonine	200,000	Negative	Negative
L-Tryptophan	200,000	Negative	Negative
L-Tyrosine	200,000	Negative	Negative
L-Valine	200,000	Negative	Negative

Structurally Unrelated Compounds

The following structurally unrelated compounds were added to drug-free, negative human urine and tested with the ARK Pregabalin II Assay. The results were evaluated both qualitatively and semiquantitatively. The compounds at the concentrations listed below were negative when tested with the ARK Pregabalin II Assay.

Compound	Concentration Tested (ng/mL)
Acetylsalicylic Acid	500,000
6-Acetyl Morphine	100,000
Amitriptyline	100,000
Amoxicillin	100,000
Amphetamine	200,000
Benzoylecgonine	100,000
Caffeine	500,000
Carbamazepine	100,000
Chlorpromazine	100,000
Cimetidine	100,000
Clomipramine	100,000
Codeine	100,000
Desipramine	100,000
Dextromethorphan	200,000
Dihydrocodeine	100,000
Doxepin	200,000
1R,2S(-) Ephedrine	100,000
1S,2R(+) Ephedrine	100,000
Fentanyl	100,000
Fluoxetine	100,000
Fluphenazine	100,000
Heroin	100,000
Hydrocodone	100,000
Hydromorphone	100,000
Imipramine	100,000
Levorphanol	50,000

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with pregabalin (± 50% of the cutoff concentration). The results were evaluated both qualitatively and semiquantitatively. No interference was observed when tested with the ARK Pregabalin II Assay.

Compound	Concentration Tested	250 ng/mL (-50% Cutoff)	750 ng/mL (+50% Cutoff)
Acetone	1000 mg/dL	Negative	Positive
Ascorbic Acid	1500 mg/dL	Negative	Positive
Bilirubin – Unconjugated	2 mg/dL	Negative	Positive
Bilirubin – Conjugated	2 mg/dL	Negative	Positive
Boric Acid	1% w/v	Negative	Positive
Creatinine	500 mg/dL	Negative	Positive
Ethanol	1000 mg/dL	Negative	Positive
Galactose	10 mg/dL	Negative	Positive
Glucose	2000 mg/dL	Negative	Positive
Hemoglobin	300 mg/dL	Negative	Positive
Human Albumin	500 mg/dL	Negative	Positive
Human Gamma Globulin	500 mg/dL	Negative	Positive
Oxalic Acid	100 mg/dL	Negative	Positive
Riboflavin	7.5 mg/dL	Negative	Positive
Sodium Chloride	6000 mg/dL	Negative	Positive
Urea	6000 mg/dL	Negative	Positive

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of pregabalin at ± 50% of the cutoff concentration. The results were evaluated both qualitatively and semiquantitatively. No interference was observed when tested with the ARK Pregabalin II Assay.

Method Comparison

A total of one hundred thirty-three (133) unaltered clinical human urine specimens that are not individually identifiable were analyzed for pregabalin with the ARK Pregabalin II Assay in both qualitative and semiquantitative modes and the results were compared to LC-MS/MS. Results are summarized in the table below.

		LC-MS/MS	
ARK Pregabalin II Assay (500 ng/mL Cutoff)		(+)	(-)
	(+)	67	0
	(-)	0	66

12 REFERENCES

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13 TRADEMARKS

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