ARK Diagnostics, Inc.

ARK™ *Levetiracetam II Assay*

This ARK Diagnostics, Inc. package insert for the ARK Levetiracetam II Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable. A Summary of Safety and Performance is available through Eudamed (European database on medical devices), SRN: US-MF-000023925.

Customer Service

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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 2797	CE Mark with notified body number
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2
Rx Only	For Prescription Use Only		

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Reagent Kit REF 5070-0001-00
5070-0001-01

1 Name

ARK™ ***Levetiracetam II Assay***

2 Intended Use

The ARK Levetiracetam II Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of levetiracetam in human serum or plasma on automated clinical chemistry analyzers. Levetiracetam concentrations can be used as an aid in management of patients treated with levetiracetam.

3 Summary and Explanation of the Test

Levetiracetam (KEPPRA®, (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide) is an anti-convulsant drug approved for use as adjunctive therapy in the treatment of epilepsy.[1]

4 Principles of the Procedure

ARK Levetiracetam II Assay is a homogeneous immunoassay based on competition between drug in the specimen and levetiracetam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. 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 the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 Reagents

REF	Product Description	Quantity/Volume
5070-0001-00	**ARK Levetiracetam II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibody to levetiracetam, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	1 X 28 mL
	Reagent R2 – Enzyme Levetiracetam labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	1 X 14 mL

REF	Product Description	Quantity/Volume
5070-0001-01	**ARK Levetiracetam II Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibody to levetiracetam, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme Levetiracetam labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	1 X 58 mL

Reagent Handling and Storage

ARK Levetiracetam 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 Levetiracetam 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**.** Laboratory professional use only.
- For prescription use only. *Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner.*
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 Specimen Collection and Preparation for Analysis

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice. A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of levetiracetam. Time of blood draw since last dose should be noted.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
    - Sodium heparin

    - Lithium heparin
    - Potassium EDTA
- **Process the blood as soon as possible after collection to prepare serum or plasma, since hydrolysis of levetiracetam may occur in the prolonged presence of whole blood.[2-3]**
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Follow the collection tube manufacturer's recommendations for collection, processing, and centrifugation.
- CLSI document GP44-A4 outlines procedures for minimizing artifacts due to specimen collection and handling for common laboratory tests.[4]
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- The presence of bubbles or foam on specimens can lead to short sample delivery and erroneous results.
- Each laboratory should consult available literature and internal data regarding specimen stability.
- Based on studies performed by ARK Diagnostics, clarified specimens may be stored up to one week at 2 to 8°C. If testing will be delayed more than one week, specimens should be stored frozen (≤ -10°C) up to four weeks prior to being tested. Care should be taken to limit the number of freeze-thaw cycles.
- **Handle all patient specimens as if they were potentially infectious.**

8 Procedure

Materials Provided

ARK Levetiracetam II Assay – **REF** 5070-0001-00 or 5070-0001-01

Materials Required – Provided Separately

ARK Levetiracetam II Calibrator – **REF** 5070-0002-00

Quality Controls – ARK Levetiracetam II Control – **REF** 5070-0003-00

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. Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Levetiracetam II Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets which have been CLIA categorized or bear the CE Mark

have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

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

Calibration
Perform a full calibration (6-point) procedure using the ARK Levetiracetam II Calibrators A, B, C, D, E, and F; run calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of 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 Levetiracetam II Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

9 Results

Report result units as µg/mL or µmol/L. To convert results from µg/mL levetiracetam to µmol/L levetiracetam, multiply µg/mL by 5.88. The levetiracetam value from this assay should be used in conjunction with other clinical information. Refer to the instrument specific operator’s manual for any result error codes.

10 Limitations of Procedure

This assay is designed for use with serum or plasma only; refer to the section **Specimen Collection and Preparation for Analysis**. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variabilities. See the section **Expected Values** below.

Brivaracetam (Briviact®)[5] interferes with measurements of levetiracetam (Keppra®) in the ARK Levetiracetam II Assay. Patients undergoing a switch in drug therapy involving Keppra and Briviact should not be monitored for levetiracetam using the ARK assay if there is a possibility these drugs are co-present in circulation.

11 Expected Values

A reference range for levetiracetam has not been well established. Reference ranges for seizure control have been proposed, which include serum/plasma trough concentrations from 6 to 46 µg/mL (35 to 270 µmol/L)[6-11] or 10 to 40 µg/mL (59 to 235 µmol/L) including a laboratory alert level at 50 µg/mL (294 µmol/L).[12] However, these ranges have not been validated by adequate controlled trials, and in general the relationship between these serum concentrations and clinical effect has not been well-defined. Levetiracetam drug concentrations should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Circulating levels of levetiracetam (serum blood concentrations) may be affected by compliance[13], renal function[14], pregnancy[15], drug-drug interactions and timing of the sample draw. Furthermore, the clinical effect of these serum blood concentrations may be further altered by changes in progression in the severity of the disease and the addition or withdrawal of concomitant drugs which may interact pharmacodynamically with circulating levels of levetiracetam.

The reference range of drug concentrations which is quoted should only imply a lower limit below which a therapeutic response is relatively unlikely to occur, and an upper limit above which toxicity is relatively likely to occur in the specific patient populations studied. Generally, clinicians using reference ranges such as these should be aware that, because of individual variation, patients may achieve therapeutic benefit with serum drug concentrations outside of these ranges and may experience toxicity with levels below the lower limit of the reference range. Sampling time should be standardized such that trough serum concentrations are measured just before the next dosage, preferably in the morning.

12 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Beckman Coulter AU680® automated clinical chemistry analyzer System.

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the ARK Levetiracetam II Assay was determined according to CLSI EP17-A and is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed (≤20% CV with ±15% recovery). The LOQ was determined to be 2.0 µg/mL, and may depend on analyzer-specific performance.

Measurement Range

The analytical measurement range of the assay is 2.0 to 100.0 µg/mL. A manual dilution protocol has not been validated to enable measurements of concentrations above the measurement range.

Report results below this range as <2.0 µg/mL or below the analyzer-specific lower LOQ established in your laboratory (whichever is higher). Report results above this range as >100.0 µg/mL or above the analyzer-specific upper LOQ established in your laboratory (whichever is lower).

Recovery

Accuracy (analytical recovery) was performed by adding concentrated levetiracetam drug into human serum negative for levetiracetam. A stock concentrate of highly pure levetiracetam was added volumetrically to human serum negative for levetiracetam, representing drug concentrations across the assay range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
2.0	1.9	95.0
4.0	3.9	97.5
10.0	9.8	98.0
20.0	20.1	100.5
45.0	46.2	102.6
80.0	77.8	97.3
100.0	100.3	100.3

Linearity

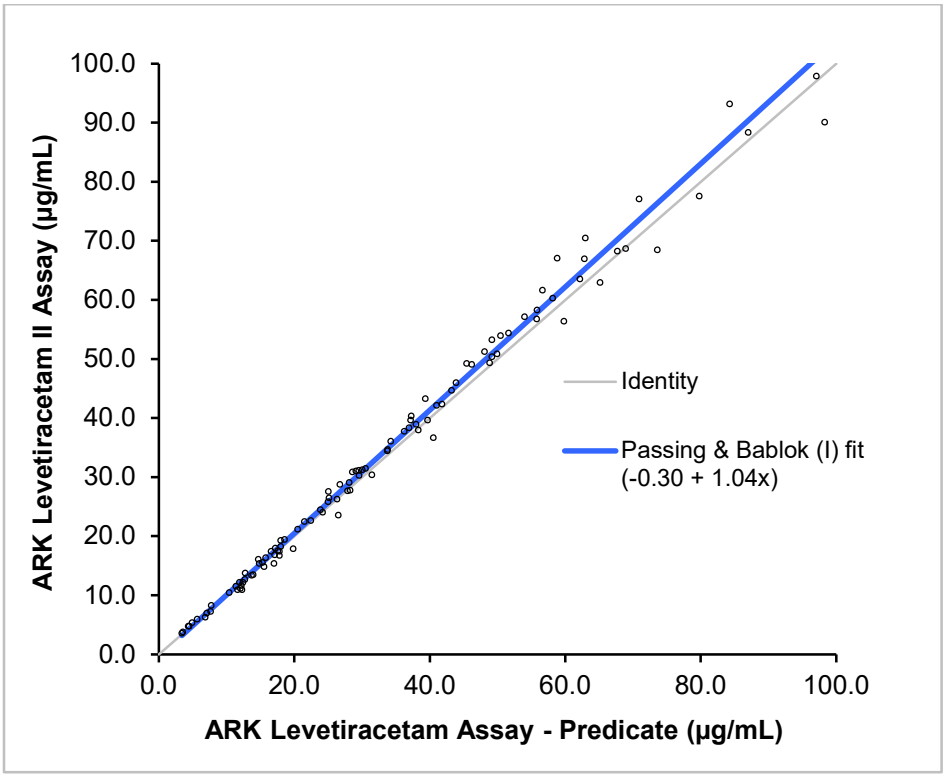
Linearity studies were performed as suggested in CLSI Protocol EP6-A. A 100.0 µg/mL serum sample was prepared and dilutions were made proportionally with human serum negative for levetiracetam. Levetiracetam concentrations ranged from 2.0 to 100.0 µg/mL. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values or ±15% below 3.0 µg/mL. A linear relationship was demonstrated between 2.0 and 100.0 µg/mL. Results are shown below.

Estimated Value (µg/mL)	Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	% Difference
0.0	0.1	NA	NA	NA
2.0	1.88	2.01	2.23	11.2
3.0	3.2	3.04	3.22	5.9
4.0	3.9	4.07	4.20	3.3
6.0	6.1	6.13	6.17	0.7
10.0	11.0	10.25	10.10	-1.5
20.0	20.0	20.56	19.84	-3.5
40.0	42.0	41.18	39.03	-5.2
60.0	62.1	61.80	57.82	-6.4
80.0	78.9	82.42	76.21	-7.5
100.0	105.4	103.03	94.20	-8.6

Method Comparison

Correlation studies were performed using CLSI Protocol EP9-A2. Results from the ARK Levetiracetam II Assay performed on the Beckman Coulter AU680 were compared with results from the predicate ARK Levetiracetam Assay performed on the Roche/Hitachi 917. Levetiracetam concentrations ranged from 3.4 µg/mL to 98.3 µg/mL. Results of the Passing-Bablok[16] regression analysis for the study are shown below (with 95% confidence limits).

Slope	1.04	(1.03 to 1.06)
y-intercept	-0.30	(-0.78 to 0.11)
Correlation Coefficient (r^2)	0.99	(0.985 to 0.993)
Number of Samples	104	



Precision

Precision was determined as described in CLSI Protocol EP5-A3. Tri-level controls and three human serum pooled specimens containing levetiracetam were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV.

			Within Run		Between Day		Total	
Sample	**N**	**Mean (µg/mL)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
ARK Levetiracetam II Control								
LOW	160	7.6	0.16	2.1	0.09	1.2	0.18	2.3
MID	160	30.5	0.45	1.5	0.34	1.1	0.60	2.0
HIGH	160	75.7	1.41	1.9	0.99	1.3	2.19	2.9
Human Serum								
LOW	160	7.7	0.11	1.4	0.06	0.8	0.12	1.6
MID	160	32.6	0.47	1.4	0.34	1.1	0.61	1.9
HIGH	160	80.5	1.62	2.0	0.80	1.0	1.79	2.2

Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A3 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of levetiracetam (approximately 15 and 50 µg/mL) were evaluated. Each sample was assayed using the ARK Levetiracetam II Assay, along with a serum control of levetiracetam. Measurement of levetiracetam resulted in ≤10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery	
Interfering Substance	**Interferent Concentration**	**15 µg/mL Levetiracetam**	**50 µg/mL Levetiracetam**
Human Albumin	12 g/dL	98.7	99.9
Bilirubin - conjugated	72 mg/dL	100.2	100.9
Bilirubin - unconjugated	72 mg/dL	101.7	97.1
Cholesterol	620 mg/dL	94.9	100.8
Human Gamma-Globulin	12 g/dL	102.7	98.7

Hemoglobin	1050 mg/dL	100.9	95.8
Rheumatoid Factor	1080 IU/mL	98.8	94.5
Triglycerides	1670 mg/dL	98.6	95.9
Uric Acid	30 mg/dL	91.0	98.8

Specificity

Levetiracetam is hydrolyzed to its major metabolite 2-pyrrolidone-*N*-butyric acid (ucb L057) and two minor metabolites.[3] Other medications routinely administered with levetiracetam and anti-epileptic drugs were also tested to determine whether these compounds affect the quantitation of levetiracetam concentrations using the ARK Levetiracetam II Assay. High levels of these compounds were spiked into serum pools containing low (15 µg/mL) and high (50 µg/mL) therapeutic levels of levetiracetam. The samples were analyzed and the levetiracetam concentrations of samples containing interferent were compared to the control serum.

Metabolites

The metabolite ucb L057 was tested for cross-reactivity.

Metabolite	ucb L057 (µg/mL)	Percent Cross-Reactivity		Percent Interference	
		Levetiracetam 15 µg/mL	Levetiracetam 50 µg/mL	Levetiracetam 15 µg/mL	Levetiracetam 50 µg/mL
ucb L057: 2-pyrrolidone-*N*-butyric acid	250.0	0.0	0.0	0.8	0.1

Drug Interference

Due to structural similarities, brivaracetam (Briviact®) crossreacts substantially in the ARK Levetiracetam II Assay. Measurements of levetiracetam should not be made with the ARK assay when both drugs are present in circulation.

Levetiracetam-selective antibody did not crossreact with other anti-epileptic or coadministered drugs tested. A high concentration of each compound was spiked into normal human serum with known levels of levetiracetam (approximately 15 and 50 µg/mL) and assayed along with a serum control of levetiracetam. Measurement of levetiracetam resulted in ≤10% error in the presence of drug compounds at the levels tested.

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Acetaminophen	500	Nortriptyline	20
Acetylsalicylic acid	1000	Oxcarbazepine	50
Amitriptyline	20	Phenobarbital	200
Caffeine	100	Phenytoin	200
Carbamazepine	120	Primidone	100
Clonazepam	50	Probenecid	600
Cyclosporin A	40	Salicylic Acid	500
Diazepam	50	Sulfamethoxazole	400
Digoxin	40	Sulfisoxazole	400
Erythromycin	200	Theophylline	250
Ethosuximide	250	Tiagabine	200
Felbamate	250	Topiramate	250
Gabapentin	100	Trimethoprim	40
Heparin	200 units/mL	Valproic Acid	500
Hydrochlorothiazide	20	Verapamil	100
Ibuprofen	500	Vigabatrin	150
Lamotrigine	250	Warfarin	250
Naproxen	500	Zonisamide	250

13 References

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14 Trademarks

ARK™ is a trademark of **ARK** Diagnostics, Inc.

Other brand or product names are trademarks of their respective holders.
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