ARK Diagnostics, Inc.

ARK™ *Levetiracetam Assay*

This ARK Diagnostics, Inc. package insert for the ARK Levetiracetam 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.

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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
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™ *Levetiracetam Assay*

2 INTENDED USE

The ARK Levetiracetam 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 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 proportional 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
5024-0001-00	**ARK Levetiracetam Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies 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

Reagent Handling and Storage

ARK Levetiracetam 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.**

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.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- 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.
- 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]
- DO NOT USE GEL SEPARATORS.
- 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.
- 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 Assay – REF 5024-0001-00

Materials Required – Provided Separately

ARK Levetiracetam Calibrator – REF 5024-0002-00
Quality Controls – ARK Levetiracetam Control – REF 5024-0003-00

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.

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 Calibrators A, B, C, D, E, and F; test 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. CAL A is the calibration blank.

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 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.

Manual Dilution Protocol

To estimate drug levels in specimens exceeding the upper limit of quantitation, manually dilute the specimen with zero calibrator (CAL A). Multiply the assayed result by the dilution factor.

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of CAL A)}}{\text{Specimen Volume}}$$

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 sections **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®)[4] interferes with measurements of levetiracetam (Keppra®) in the ARK Levetiracetam Assay. Patients undergoing a switch in drug therapy involving Keppra and Briviact should not be monitored for levetiracetam using the ARK assay. Serum levels of levetiracetam and/or brivaracetam should be confirmed by a valid chromatographic method 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. Tentative reference ranges for seizure control have been proposed, which include concentrations from 6 to 46 µg/mL (35 to 270 µmol/L; trough samples). 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.[5-9] 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, renal function, pregnancy, 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 Roche/Hitachi 917 System.

Sensitivity

Limit of Quantitation (LoQ)

The LOQ of the ARK Levetiracetam 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.

Assay Range

The range of the assay is 2.0 to 100.0 µg/mL. Report results below this range as <2.0 µg/mL or below the analyzer-specific lower LOQ established in your laboratory. Report results above this range as >100.0 µg/mL or above the analyzer-specific upper LOQ established in your laboratory.

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.

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

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percentage Recovery
2.0	1.9	95.8
4.0	3.8	94.6
10.0	10.0	100.0
20.0	19.2	95.9
45.0	44.1	98.0
80.0	79.3	99.1
100.0	105.3	105.3

Linearity

Linearity studies were performed as suggested in CLSI/NCCLS 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 1.0 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
2.0	1.9	2.1	2.4	13.2
3.0	3.2	3.1	3.4	7.6
4.0	4.1	4.2	4.3	4.8
5.0	5.3	5.2	5.3	3.1
6.0	6.4	6.2	6.3	2.0
7.0	7.6	7.2	7.3	1.3
8.0	8.4	8.3	8.3	0.7
9.0	9.5	9.3	9.3	0.3
10.0	10.7	10.3	10.3	-0.1
20.0	20.7	20.6	20.4	-1.3
30.0	31.0	31.0	30.5	-1.4
40.0	41.3	41.3	40.8	-1.2
50.0	51.9	51.6	51.1	-0.9
60.0	60.3	61.9	61.6	-0.5
70.0	71.2	72.2	72.1	-0.1
80.0	81.4	82.5	82.8	0.3
90.0	93.7	92.8	93.5	0.7
100.0	104.6	103.1	104.3	1.2

Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Levetiracetam Assay were compared with results from LC/MS/MS. The levetiracetam concentrations ranged from 2.0 µg/mL to 86.4 µg/mL. Results of the Passing-Bablok[10] regression analysis for the study are shown below (with 95% confidence limits).

Slope	1.01	(0.99 to 1.03)
y-intercept	0.25	(- 0.24 to 0.63)
Correlation Coefficient (r2)	0.97	(0.96 to 0.97)
Number of Samples	305	

Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. 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.

Sample	N	Mean (µg/mL)	Within Run		Between Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Levetiracetam Control								
LOW	160	7.5	0.25	3.4	0.23	3.2	0.34	4.5
MID	160	29.4	0.85	2.9	0.83	2.8	1.08	3.7
HIGH	160	73.4	2.14	2.9	2.03	2.8	3.08	4.2
Human Serum								
LOW	160	6.9	0.26	3.8	0.22	3.1	0.33	4.8
MID	160	30.2	0.87	2.9	1.10	3.7	1.23	4.1
HIGH	160	75.5	2.19	2.9	2.35	3.1	3.31	4.4

Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 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 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.

Interfering Substance	Interferent Concentration	Percentage Recovery	
		15 µg/mL Levetiracetam	50 µg/mL Levetiracetam
Albumin	12 g/dL	99.8	102.6
Bilirubin - conjugated	70 mg/dL	100.4	102.1
Bilirubin - unconjugated	70 mg/dL	99.3	107.9
Cholesterol	535 mg/dL	105.3	94.0
Gamma-Globulin	12 g/dL	99.8	109.5
Hemoglobin	1000 mg/dL	98.6	100.9
Intralipid®	1500 mg/dL	97.1	99.8
Rheumatoid Factor	1100 IU/mL	98.1	106.4
Triglycerides	1033 mg/dL	96.8	100.2
Uric Acid	30 mg/dL	99.6	102.5

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 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	-0.2	1.3	-3.0	6.6

Drug Interference

Due to structural similarities, brivaracetam (Briviact®) crossreacts substantially in the ARK Levetiracetam 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	Conc. Tested (µg/mL)	Percentage Recovery	
		15 µg/mL Levetiracetam	50 µg/mL Levetiracetam
Acetaminophen	200	99.3	97.5
Acetylsalicylic acid	1000	103.2	98.9
Amitriptyline	20	98.4	100.7
Caffeine	100	95.4	97.7
Carbamazepine	120	101.1	99.7
Clonazepam	50	100.2	100.4
Cyclosporin A	40	99.9	98.4
Diazepam	50	100.3	98.6
Digoxin	40	92.9	100.2
Erythromycin	200	99.0	97.9
Ethosuximide	250	98.1	101.1
Felbamate	250	100.8	97.9
Gabapentin	100	101.3	96.3
Heparin	200 units/mL	97.0	97.2
Hydrochlorothiazide	20	98.2	98.9
Ibuprofen	500	98.5	99.2
Lamotrigine	250	94.3	102.4
Naproxen	500	99.0	101.3
Nortriptyline	20	99.3	97.8
Oxcarbazepine	50	95.5	100.4
Phenobarbital	200	98.8	99.4
Phenytoin	200	97.8	96.8
Primidone	100	97.7	97.3
Probenecid	600	100.5	101.5
Salicylic Acid	500	95.1	98.4
Sulfamethoxazole	400	97.9	96.3
Sulfisoxazole	400	100.6	100.4
Theophylline	250	96.6	101.1
Tiagabine	200	99.0	97.5
Topiramate	250	94.7	99.2
Trimethoprim	40	102.0	99.3
Valproic Acid	500	98.7	96.2
Verapamil	100	100.3	96.4
Vigabatrin	150	94.0	97.1
Warfarin	250	96.6	102.3
Zonisamide	250	100.3	101.7

13 REFERENCES

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