ARK Diagnostics, Inc.

ARK *T4 Assay*

This ARK Diagnostics, Inc. package insert for the ARK T4 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.

Customer Service

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
	Consult Instructions for Use		Temperature limitation
R1 R2	Reagent 1/ Reagent 2		

Reagent Kit REF 5027-0001-00

Reagent Kit REF 5027-0001-01

Reagent Kit REF 5027-0001-02

1 Name

ARK™ ***T4 Assay***

2 Intended Use

The ARK T4 Assay is a homogeneous immunoassay intended for the quantitative determination of T4 in canine and feline serum. The assay is designed for use in laboratories with automated clinical chemistry analyzers. For veterinary use only.

3 Summary and Explanation of the Test

T4 is a vital hormone produced by the thyroid gland in animals, including humans. In veterinary medicine, T4 plays a crucial role in regulating the metabolic rate of the body, affecting various physiological processes such as growth, development, and metabolism. It helps in maintaining the proper functioning of various organ systems, including the nervous, cardiovascular, and digestive systems.[1–3]

When there is an imbalance in T4 levels, it can lead to various health issues in animals. Hypothyroidism, a condition characterized by insufficient production of thyroid hormones, including T4, can result in symptoms such as lethargy, weight gain, poor coat quality, and intolerance to cold temperatures. On the other hand, hyperthyroidism, which involves an overproduction of thyroid hormones, can lead to symptoms like weight loss, increased appetite, restlessness, and an increase in heart rate. [1–3]

Most of the T4 in blood is bound to various carrier proteins such as albumin, prealbumin, and thyroxine-binding globulin (TBG).[4]

4 Principles of the Procedure

The ARK T4 Assay is a homogeneous immunoassay using ready-to-use liquid reagents. The assay uses 8-anilino-1-napthalene sulfonic acid (ANS) to dissociate T4 from the plasma binding proteins. The dissociated T4 in the sample is allowed to compete with enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled T4 for binding to the antibody. As the latter binds antibody, enzyme activity decreases. In the presence of T4 from the specimen, enzyme activity increases and is directly related to the T4 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
5027-0001-00	**ARK T4 Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to T4, 8-anilino-1-napthalene sulfonic acid (ANS), glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	1 X 115 mL
	Reagent R2 – Enzyme T4 labeled with bacterial rG6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	1 X 58 mL

REF	Product Description	Quantity/Volume
5027-0001-01	**ARK T4 Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to T4, 8-anilino-1-napthalene sulfonic acid (ANS), glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	4 X 28 mL
	Reagent R2 – Enzyme T4 labeled with bacterial rG6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	4 X 14 mL

REF	Product Description	Quantity/Volume
5027-0001-02	**ARK T4 Assay** **Reagent R1 – Antibody/Substrate** Rabbit monoclonal antibodies to T4, 8-anilino-1-napthalene sulfonic acid (ANS), glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers	4 X 115 mL
	Reagent R2 – Enzyme T4 labeled with bacterial rG6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	4 X 58 mL

Reagent Handling and Storage

ARK T4 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 T4 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 veterinary 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 is required.
- Whole blood cannot be used.
- Follow the collection tube manufacturer's recommendations for collection, processing and centrifugation.
- 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.
- 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 T4 Assay – **REF** 5027-0001-00, 5027-0001-01 or 5027-0001-02

Materials Required – Provided Separately
ARK T4 Calibrator – **REF** 5027-0002-00, 5027-0002-01
Quality Controls – ARK T4 Control – **REF** 5027-0003-00

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 T4 Assay, available from your distributor or ARK Customer Service. 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 T4 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.

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 T4 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 T4 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.

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

9 Results

Report result units as µg/dL.

10 Limitations of Procedure

This assay is designed for use with serum 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.

11 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer.

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the ARK T4 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 0.5 µg/dL, and may depend on analyzer-specific performance.

Assay Range

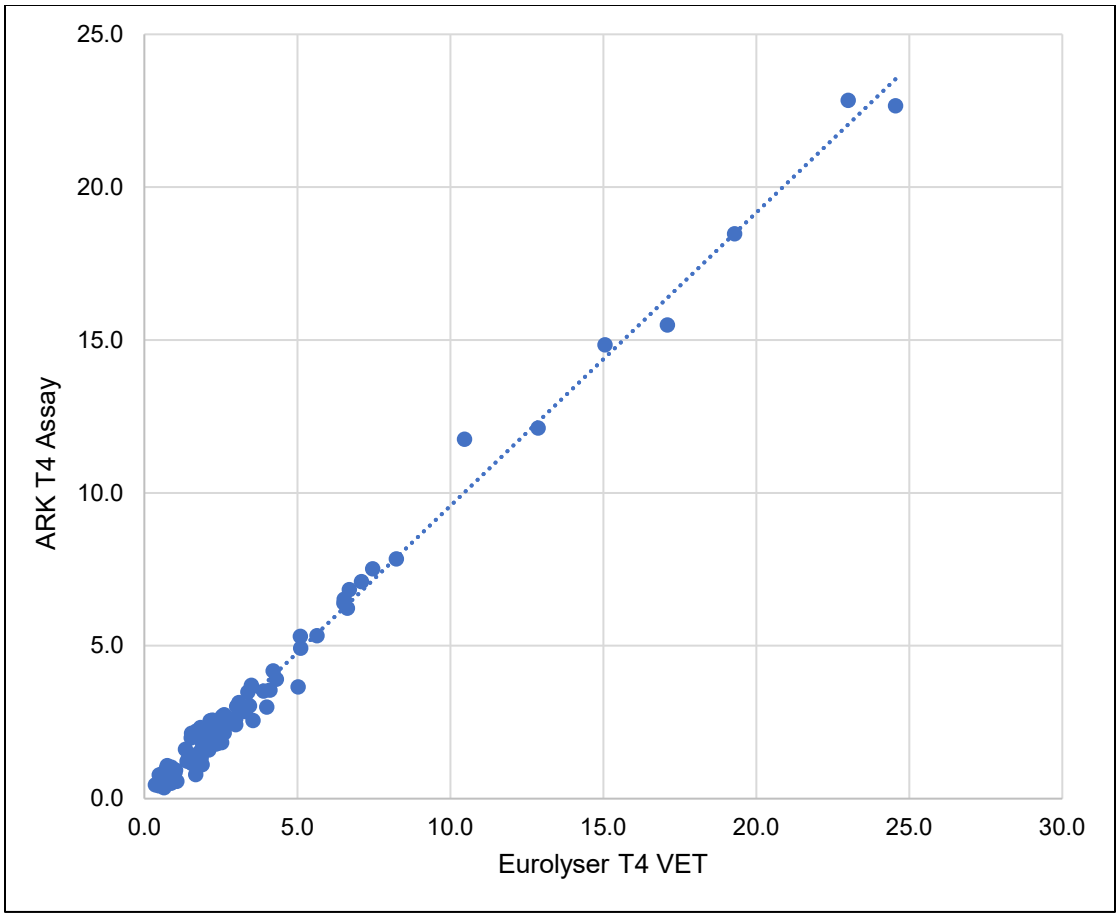
The range of the assay is 0.5 to 20.0 µg/dL. Report results below this range as <0.5 µg/dL or below the analyzer-specific lower LOQ established in your laboratory. Report results above this range as >20.0 µg/dL or above the analyzer-specific upper LOQ established in your laboratory.

Method Comparison

Correlation studies were performed using CLSI Protocol EP9-A2. A total of 50 canine, 30 feline, and 30 equine serum specimens were analyzed by the ARK T4 Assay and the Eurolyser T4 VET test on the Eurolyser CUBE-VET analyzer.

Results of the Passing-Bablok regression analysis for the study are shown below.

Slope	0.9595
y-intercept	0.0208
Correlation Coefficient (r^2)	0.99
Number of Samples	109



Precision

Precision was determined as described in CLSI Protocol EP5-A2. Quad-level controls containing T4 were used in the study. Each level was assayed in quadruplicate twice a day for 5 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 (µg/dL)	**N**	**Mean (µg/dL)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1.5	40	1.4	0.04	2.9	0.03	2.1	0.05	3.6
8.0	40	7.9	0.12	1.5	0.20	2.5	0.23	2.9
16.0	40	15.8	0.36	2.3	0.22	1.4	0.45	2.8

Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A3 as a guideline. High concentrations of the following potentially interfering substances with known levels of T4 (approximately 8 µg/dL) were evaluated. Each sample was assayed using the ARK T4 Assay. Measurement of T4 resulted in ≤15% error in the presence of interfering substances at the levels tested.

Interfering Substance	Interferent Concentration	Percentage Recovery
Bilirubin	30 mg/dL	97.8
Cholesterol	400 mg/dL	103.0
Hemoglobin	800 mg/dL	91.8
Triglycerides	1000 mg/dL	104.3

Specificity

Structurally similar compounds were tested for cross-reactivity. Triiodothyronine cross-reactivity was determined to be 10%.

Compound	Concentration Tested (µg/dL)	Mean (µg/dL)	Percent Cross-Reactivity
Triiodothyronine (T3)	10	1	10
Triiodothyroacetic acid	10	0	0
Tetraiodothyroacetic acid	1000	0	0
3-Iodotyrosine	1000	0	0
3,5-Diiodo-4-hydroxyphenylpropionic acid	1000	0	0
3,5-Diisopropyl-4-hydroxy benzoic acid	1000	0	0

12 References

1. Iturriaga MP, Cocio JA, Barrs VR. Cluster of cases of congenital feline goitrous hypothyroidism in a single hospital. J Small Anim Pract. 2020 Nov;61(11):696-703.

2. Panciera DL. Is it possible to diagnose canine hypothyroidism? J Small Anim Pract. 1999 Apr;40(4):152-7.

3. Hegstad-Davies, R. L., Torres, S. M. F., Sharkey, L. C., Gresch, S. C., Muñoz-Zanzi, C., & Davies, P. (2015). Breed-specific reference intervals for assessing thyroid function in seven dog breeds. Journal of Veterinary Diagnostic Investigation, 27(6), 716-727.

4. Mahendhar, R., Shahbaz, A., Riaz, M., Aninyei, M., Reich, D., & Sachmechi, I. (2018). Effect of albumin polymorphism on thyroid hormones: a case report and literature review. Cureus.

13 Trademarks

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