



EAGLE
BIOSCIENCES

Canine Thymidine Kinase ELISA Assay Kit

Catalog Number:

TK149-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 4.1

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INTENDED USE

The Eagle Biosciences Canine Thymidine Kinase ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative analysis of Thymidine Kinase in Canine (dog) biological samples. The Eagle Biosciences Canine Thymidine Kinase ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

Thymidine kinase (TK), particularly the cytosolic isoform TK1, is a key enzyme involved in the pyrimidine salvage pathway, catalyzing the phosphorylation of thymidine to thymidine monophosphate—a necessary step for DNA synthesis during cell proliferation. In canines, TK1 serves as a proliferation biomarker, especially sensitive to changes in cell turnover. Its levels are typically low in resting (non-dividing) cells but rise sharply during the G1 to S phase transition, making it a valuable indicator of mitotic activity. Importantly, TK1 is released into circulation during increased cellular proliferation or lysis, allowing its measurement in serum or plasma. This dynamic makes TK1 an attractive candidate for non-invasive monitoring of proliferative diseases in dogs, particularly hematologic malignancies such as lymphoma and leukemia.

Clinically, canine serum TK1 (cTK1) is increasingly used as a diagnostic and prognostic tool. Elevated cTK1 levels have been shown to correlate with both the presence and aggressiveness of neoplastic diseases, distinguishing between malignant and benign conditions with moderate to high specificity. In veterinary oncology, TK1 assays assist in early cancer detection, disease staging, and therapy monitoring, as levels often decrease with successful treatment and rise again with relapse. Research-wise, TK1 is being explored for its utility in evaluating novel anti-cancer therapies, understanding tumor biology, and as part of multi-analyte panels to improve diagnostic accuracy. Its integration with other biomarkers such as C-reactive protein or haptoglobin has shown promise in enhancing sensitivity and specificity for cancer detection in dogs, paving the way for more precise and personalized veterinary care.

PRINCIPLE OF THE ASSAY

In this assay, the thymidine kinase (TK1) present in samples reacts with the anti-TK1 antibodies which have been adhered to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-TK1, is added and complexes are formed. Following a wash step, the horseradish peroxidase (HRP) conjugated Streptavidin is added, and complexes are formed. After another wash step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of TK1 in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of TK1 in the test sample. The quantity of TK1 in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.3 sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.

- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum Samples: Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

Plasma Samples: Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

Urine Samples: Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

**Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction and is a known interfering substance.*

Specimen Pre-Treatment

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum Samples: Recommended starting dilution is 1:2. To prepare a 1:2 dilution of a sample, transfer 300 µL of sample to 300 µL of 1x diluent. This give you a 1:2 dilution.

Plasma Samples: Recommended starting dilution is 1:2. To prepare a 1:2 dilution of a sample, transfer 300 µL of sample to 300 µL of 1x diluent. This give you a 1:2 dilution.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microplate washer/aspirator
- Distilled or Deionized H₂O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

REAGENTS PROVIDED

1. Microplate

Contents: One antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format: Ready to Use
Storage: 2-8°C
Stability: Stable until the expiry date printed on the label

2. Detection Antibody (100x)

Contents: One bottle containing concentrated affinity purified antibody conjugated with biotin in a stabilizing buffer
Format: Concentrated; Requires Preparation
Volume: 150 µL/bottle
Storage: 2-8°C
Stability: Stable until the expiry date printed on the label
Preparation of
Working
Solution: **Dilute 1:100** in 1x diluent before use (e.g., 10 µL of antibody concentration in 990 µL of 1x diluent for each test strip). Dilute immediately before use and protect from light.
Mix uniformly, but gently to avoid foaming.

3. HRP Streptavidin Concentrate (100x)

Contents: One bottle containing concentrated horseradish peroxidase conjugated streptavidin in a stabilizing buffer.
Format: Concentrated; Requires Preparation
Volume: 150 µL/bottle
Storage: 2-8°C
Stability: Stable until the expiry date printed on the label

Preparation of Working Solution: **Dilute 1:100** in 1x diluent before use (e.g., 10 μ L of HRP concentration in 990 μ L of 1x diluent for each test strip). Dilute immediately before use and protect from light. Mix uniformly, but gently to avoid foaming.

4. Standard Concentrate

Contents: One bottle of lyophilized standard to be prepared for calculation of your standard curve.

Format: Lyophilized, requires preparation

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Preparation: Use immediately, or aliquoted and frozen once reconstituted and not using entire plate.

Preparation of Working Solution: Refer to the Certificate of Analysis (CoA). Working standard solution should be prepared immediately prior to use.

5. Diluent Concentrate (5x)

Contents: One bottle containing a concentrated diluent buffer

Format: Ready to Use

Volume: 50 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Preparation: Stable for one week

Preparation of Working Solution: **Dilute 1:5** with distilled or deionized water. 1 part concentrate with 4 parts water.

6. TMB Substrate

Contents: One bottle containing 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in citric acid buffer at pH 3.3

Format: Ready to Use

Volume: 12 mL/bottle

Storage: 2-8°C. Protect from light

Stability: Stable until the expiry date printed on the label

7. Stopping Solution

Contents: One bottle containing 0.3 M sulfuric acid.

Format: Ready to Use

Volume: 12 mL/bottle

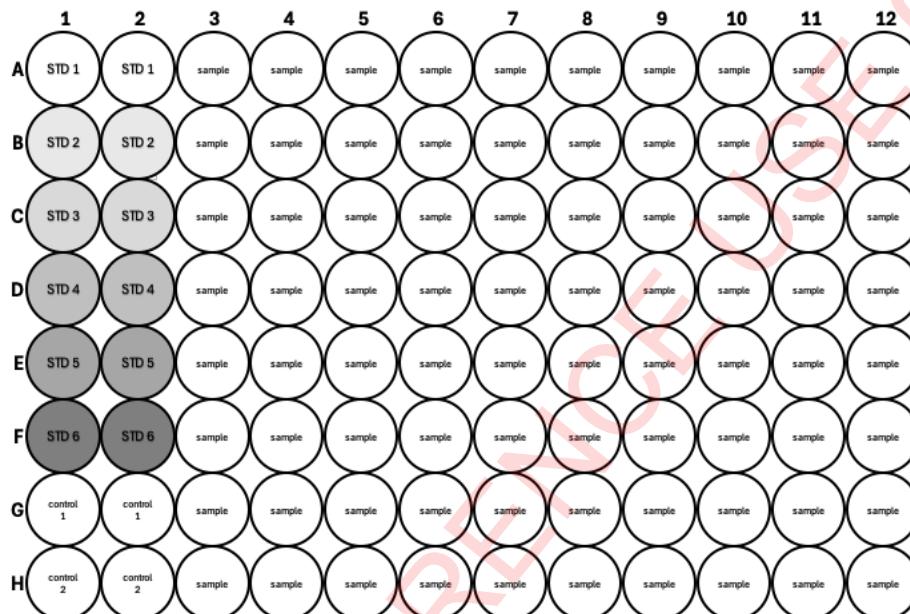
Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

8. Wash Buffer Concentrate (20x)

Contents:	One bottle containing a concentrated wash solution.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until expiry date printed on the label. After Preparation: Stable for one week.
Preparation of Working Solution:	Dilute 1:20 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 950 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT*



*Layout subject to change based on standard and control quantities

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Pipette 100 μ L of standards and samples (in duplicate) into predesignated wells
2. Incubate the microplate at room temperature for 60 minutes. Keep plate covered and level during incubation
3. Following incubation, aspirate the contents of the wells.
4. Completely fill each well with appropriately diluted wash solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
5. Pipette 100 μ L of appropriately diluted Detection Antibody to each well. Incubate at room temperature for twenty (20 \pm 2) minutes. Keep plate covered in the dark and level during incubation.

6. Wash and blot the wells as described in Step 4
7. Pipette 100 μ L of appropriately diluted HRP-streptavidin to each well. Incubate at room temperature for twenty (20 \pm 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Step 4
9. Pipette 100 μ L TMB Substrate solution into each well
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After 10 minutes, add 100 μ L of Stop Solution to each well
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specification.

CALCULATIONS

1. Subtract the average background value (average absorbance reading of standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the TK1 concentration in original samples.

WARRANTY INFORMATION

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.