INTRODUCTION

Thymidine kinase 1 (TK1)) is a cytosolic enzyme that is involved in DNA synthesis. It exists in dimeric and tetrameric forms. Levels increase in proliferating cells. It serves as a serum biomarker for some cancers. In studies at Life Diagnostics, we found elevated levels in dogs and cats with lymphoma.

PRINCIPLE OF THE ASSAY

The assay uses an anti-human TK1 monoclonal antibody developed at Life Diagnostics. We have demonstrated that it cross-reacts with human, cat, and dog TK1. Unconjugated antibody is used to coat plates and HRP-conjugated antibody is used for detection. Standards and diluted samples (100 µl) are incubated in anti-TK1 coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µl) is added and incubated for 45 minutes. If TK1 molecules are present, they are sandwiched between the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If TK1 is present, a blue color develops. Color development is stopped after 20-minutes by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of TK1 is proportional to absorbance and is derived from a standard curve.

MATERIALS

Materials provided with the kit:

- Anti-TK1 coated plate (12 x 8-well strips)
- Anti-TK1 HRP Conjugate Stock, 1 vial
- TK1 stock, 2 vials. **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Store the standard stock vial at -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate[1]. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µl aliquots to the wells of the antibody-coated plate.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

The Wash Solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water. Unused wash buffer may be stored at 4°C for one week.

DILUENT

The diluent is provided ready for use. DO NOT substitute other buffers.

STANDARD

1. The stock is lyophilized. Reconstitute it with deionized water as described on the vial label and gently mix. Prepare the 10 ng/ml standard as described on the label.
2. Label seven polypropylene tubes as 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 10 ng/ml TK1 standard into the tube labeled 5 ng/ml and mix. This provides the 5 ng/ml TK1 standard.
4. Similarly prepare the 2.5 – 0.156 ng/ml standards by two-fold serial dilution.

IMPORTANT – Use the standards within 30 minutes of stock reconstitution.

HRP CONJUGATE

The HRP conjugate is provided as a concentrated stock. Prior to use, dilute it with room temperature equilibrated YD50-1 as described on the stock vial label.

[1] Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

SAMPLES

The assay is intended for measurement of TK1 in serum or plasma. We found that a dilution of 10-fold worked well for most samples, but optimal dilutions should be determined by the end user. A ten-fold dilution can be obtained by mixing 25 µl of sample with 225 µl of YD50-1.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µl of standards and samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
5. Dispense 100 µl of HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45-minutes.
7. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
8. If necessary, strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm[2] with a plate reader within 5 minutes.

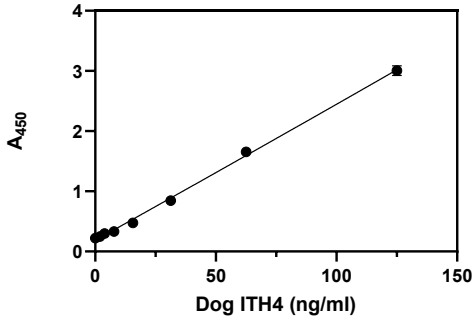
RESULTS

1. Using curve fitting software, graph the absorbance values of the standards on the Y-axis versus TK1 concentration on the X-axis.
2. Fit the curve to a second order polynomial (quadratic) equation and derive the concentration of TK1 in the diluted samples.
3. Multiply derived values by the dilution factor(s) to determine TK1 concentration in the original sample.
4. If the absorbance values of diluted samples fall outside the standard curve, samples should be further diluted and re-tested.

TYPICAL STANDARD CURVE

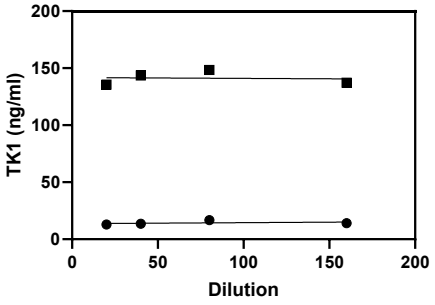
A typical standard curve is shown below. This curve is for illustration only.

TK1 (ng/ml)	A_{450}
10	3.007
5	1.654
2.5	0.845
1.25	0.474
0.625	0.333
0.313	0.298
0.156	0.250
0	0.223



PERFORMANCE

Linearity: To assess the linearity of the assay, dog serum samples with TK1 concentrations of 14.3 and 151 ng/ml were diluted with to give values within range of the assay.



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For technical assistance please email us: techsupport@lifediagnostics.com

[2] If absorbance of the high standard is ≥4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.

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