CAT THYMIDINE KINASE 1 SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: TK1-SP-8

INTRODUCTION

Thymidine kinase 1 (TK1) is an enzyme involved in DNA synthesis. Its expression levels increase in tumors that are proliferating. Upon tumor cell death and lysis, TK1 is released into serum. In cats, TK1 is a potential biomarker for lymphoma.[1]

PRINCIPLE OF THE ASSAY

The TK1 SPARCL™[a] (Spatial Proximity Analyte Reagent Capture Luminescence)[2] assay uses HRP and acridan conjugates of a TK1 monoclonal antibody developed by Life Diagnostics, Inc. When the HRP and acridan conjugated antibodies bind to TK1 they are brought into molecular proximity. With the addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies distant from HRP produce no signal. This principle allows the development of a homogeneous assay that allows rapid measurement of TK1.

The HRP and acridan conjugated antibodies are mixed with standards and diluted samples in wells of the white 96-well SPARCL™ plate[b]. After incubation for 30 minutes on a shaker at 25°C and 150 rpm, the plate is placed into a luminometer. Trigger solution containing hydrogen peroxide is injected into each well and luminescence is immediately measured. The concentration of TK1 is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-TK1 HRP conjugate **Store ≤ -70°C**
- Anti-TK1 acridan conjugate **Store ≤ -70°C**
- TK1 stock **Store ≤ -70°C**
- Diluent; CSD50-1, 2 x 50 ml
- Trigger solution; TS7-1, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

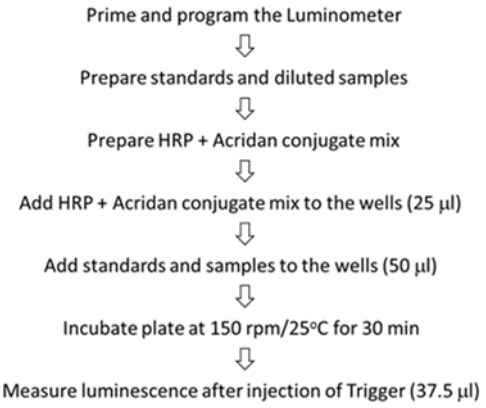
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Graphing software

STORAGE

Store the HRP conjugate, acridan conjugate and TK1 stock at or below -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. Please take the time to completely read all instructions before starting your assay. Contact us if you need clarification.
2. All reagents used in the assay should be allowed to reach room temperature (25°C) before use.
3. It is important that standards and samples be added to the SPARCL™ plate quickly. If testing large numbers of samples, rather than pipetting standards and samples directly into the white SPARCL™ plate using a single channel pipettor, we recommend the following. First, pipette an excess volume of standards and samples into appropriate wells of the clear 96-well plate. Then use an 8- or 12-channel multipipettor to quickly and efficiently transfer 50 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate hold a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The stock is comprised of pure TK1 in a carrier protein matrix. Thaw the stock just before use.

1. Label 8 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ng/ml.
2. In the tube labelled 10 ng/ml prepare the 10 ng/ml standard as detailed on the TK1 stock label.
3. Dispense 150 µl of diluent CSD50-1 into the tubes labelled 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ng/ml
4. Pipette 150 µl of the 10 ng/ml TK1 standard into the tube labeled 5 ng/ml and mix. This provides the 5 ng/ml TK1 standard.
5. Similarly prepare the remaining standards by two-fold serial dilution.

Use the standards within one hour of preparation. Store unused stock at -70°C if future use is intended.

SAMPLE PREPARATION

Serum, plasma (lithium-heparin or K3 EDTA) and ascites fluids can be tested with the assay. To avoid matrix effects samples must be diluted at least 40-fold with CSD50-1. Optimum dilutions must be determined by the end user. Hemolyzed samples should not be tested because false low values may be obtained.

[a]The SPARCL technology was developed by Lumigen Corp.

[b]The plate has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. If necessary, after thawing, briefly centrifuge to ensure that the contents are at the bottom of the vials. Prepare the mix shortly before use using the CSD50-1 diluent provided with the kit.

LUMINOMETER SETUP

1. The luminometer must be capable of injection and simultaneous measurement of luminescence without any delay.
2. Prime the luminometer injection port with 1 ml of trigger solution.
3. Place the injection needle into the injection port as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 µl of trigger solution per well and to measure from time zero for 1 second (50 x 0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as 12 x 8-well strips, allowing use of fewer than 96-wells, make sure that the luminometer is programmed to inject trigger solution only into the wells being used.
7. We use a BMG LUMIstar Omega set at a gain of 3600. Optimal gain should be determined by the end user.
8. There are a number of manufacturers of luminometers that are equipped to run a SPARCL™ assay. Please contact Life Diagnostics or Lumigen (www.lumigen.com) to discuss your luminometer.

PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with trigger solution and that the injection needle is positioned in the injection port.
2. Secure the desired number of SPARCL™ 8-well strips in the holder. Immediately seal unused strips in the resealable bag with desiccant and antioxidant. Store unused strips at 2-8°C.
3. Aliquot 25.0 µl of conjugate mix into each well.
4. Dispense 50.0 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
5. Incubate on an orbital micro-plate shaker at 150 rpm 25°C for 30 minutes.
6. After the 30-minute incubation, place the plate in the luminometer and measure luminescence after injection of trigger solution (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

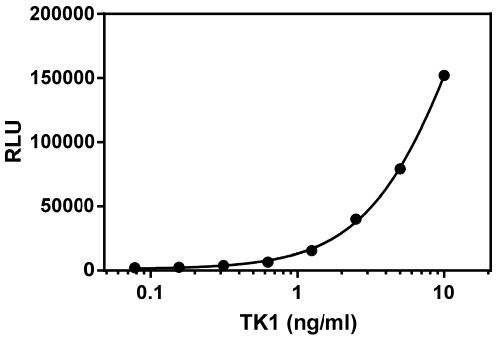
CALCULATION OF RESULTS

1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells. We routinely use the sum of RLU values from a 100-980 ms data collection window.
2. Determine the sum of RLU values within the data collection window for the standards and samples.
3. Using graphing software, construct a standard curve by plotting the sum of the RLU values for the standards versus the $\log_{10}$ of the TK1 concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of TK1 in the samples from the standard curve (remember to derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the concentration of TK1 in the original sample.
6. If the sum of the RLU values of diluted samples fall outside the standard curve, samples should be appropriately diluted and re-tested

TYPICAL STANDARD CURVE

A typical standard curve with sum of RLU plotted on the Y-axis versus TK1 concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. A standard curve must be run for each experiment.

TK1 (ng/ml)	RLU
10	152062
5	79229
2.5	40165
1.25	15559
0.625	6661
0.313	3972
0.156	2664
0.078	2284



ASSAY PERFORMANCE

We found TK1 levels ranging from 0 to 95 ng/ml in serum from cats reported as sick by veterinarians. Dilutional linearity was obtained if samples were diluted 40-fold or greater.

REFERENCES

1. Taylor SS. et al. Serum thymidine kinase activity in clinically healthy and diseased cats: a potential biomarker for lymphoma. Journal of Feline medicine and Surgery. 15(2):142-147 (2012)
2. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. J Am Chem Soc. 20;135(11):4191-4 (2013)

Rev 012218

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