DOG IgM SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: IGM-SP-4

PRINCIPLE OF THE ASSAY

The dog IgM SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two affinity purified IgM μ-chain specific antibodies. One is conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated IgM antibodies bind to IgM they are brought into close 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 determination of IgM concentrations.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well white SPARCL™ plate provided with the kit[2]. 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 IgM is proportional to luminescence and is derived from a standard curve.

Because the dog IgM SPARCL assay uses a homogenous format, a prozone or hook effect occurs at high IgM concentrations. For this reason, and to avoid matrix effects, serum or plasma samples should initially be tested at dilutions of 16,000 fold. When measuring IgM in other biological fluids or extracts, samples should be tested at a series of dilutions in order to ensure that false low values are not obtained due to either prozone or matrix effects.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-dog IgM HRP stock. **Store ≤ -70°C**
- Anti-dog IgM acridan stock. **Store ≤ -70°C**
- IgM stock. **Store ≤ -70°C**
- Diluent (CSD50-1, 2 bottles)
- Trigger solution (TS7-1)
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Micro-Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC graphing software

STORAGE

Store the HRP and acridan conjugates at or below -70°C. 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.

GENERAL INSTRUCTIONS

1. Please take the time to completely read all of the 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 a large number 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 have a maximum volume of 300 μl.

STANDARD PREPARATION

The IgM stock is comprised of dog serum of known IgM content diluted in a stabilizing carrier protein matrix.

1. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml[3].
2. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 250 ng/ml standard.
3. Dispense 150 μl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
4. Pipette 150 μl of the 250 ng/ml IgM standard into the tube labeled 125 ng/ml and mix. This provides the 125 ng/ml IgM standard.
5. Similarly prepare the remaining standards by two-fold serial dilution.

Please Note: If future use of the reconstituted IgM stock is intended, it should be stored or below -70°C.

SAMPLE PREPARATION

We found that IgM was present in dog serum at a concentration of 1.54±0.17 mg/ml (mean±SD, n=9). In order to obtain values within range of the standard curve and to avoid matrix effects we suggest that samples initially be tested at a dilution of 16,000 fold. This can be accomplished as follows.

1. For each sample to be tested. dispense 247.5 μl and 318 μl of diluent into two separate tubes.
2. Aliquot 2.50 μl of serum or plasma into the first tube containing 247.5 μl of diluent and mix to give a 100-fold dilution of the sample.
3. Mix 2.0 μl of the 100-fold diluted sample with 318 μl of diluent in the second tube to give an 16,000-fold dilution.

Do not test serum or plasma at dilutions less than 8,000-fold.

CONJUGATE MIX PREPARATION

[1] The SPARCL™ technology was developed by Lumigen Corp.

[2] The white SPARCL™ plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

[3] Dilutions of standards can be performed in wells A1-A8 of the clear untreated 96-well plate provided with the kit. This allows rapid transfer of standards to the white SPARCL™ plate using a multipipettor

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Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. Prepare the mix shortly before use using the diluent provided with the kit (CSD50-1).

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 at least 1 ml of trigger solution.
3. Place the injection needle into the injection port, (necessary 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 a 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.

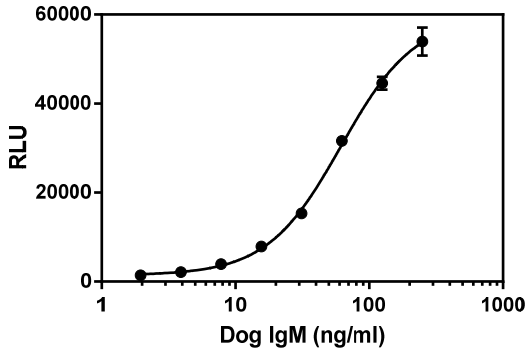
CALCULATION OF RESULTS

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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the $\log_{10}$ of the IgM concentration in ng/ml.
3. Fit the data using a variable slope, four-parameter logistic curve.
4. Derive the corresponding concentration of IgM in the samples from the standard curve (derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgM in the serum or plasma sample.
6. If the RLU values of diluted samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with RLU plotted on the Y-axis versus $\log_{10}$ IgM 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 with each experiment.

IgM (ng/ml)	RLU
250	53901
125	44553
62.5	31607
31.25	15309
15.63	7859
7.81	3916
3.91	2108
1.95	1422



REFERENCES

1. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. J Am Chem Soc. 20;135(11):4191-4 (2013)

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For technical assistance please email us at
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