INTRODUCTION

S100A8 and S100A9 are calcium binding proteins that are expressed in neutrophils, monocytes and macrophages. They exist as heterodimers and homodimers, although the heterodimer, known as calprotectin, predominates. S100 A8 and A9 are released during inflammatory disease; calprotectin is used as a serum biomarker of sepsis and a fecal biomarker of IBD in humans.

PRINCIPLE OF THE ASSAY

The dog S100A8 assay uses SPARCL™[1] technology. Two S100A8 antibody conjugates are used; one to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugates bind to S100A8 they are brought into proximity. Upon addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of luminescence that is proportional to S100A8 concentration.

Diluted serum or plasma samples and standards (100 µl) are dispensed into test tubes and mixed with 0.5 ml of combined acridan and HRP conjugates. After 45 minutes, tubes are placed in the VetBio-1 luminometer. Luminescence is measured after injection of background reducer (50 µl), that eliminates nonspecific luminescence, followed by trigger-solution containing hydrogen peroxide (100 µl). The concentration of S100A8 is determined from the ratio of sample luminescence to the that of the 10 ng/ml standard.

Prime and program the VetBio-1 luminometer
⇩
Dilute samples
⇩
Prepare 10 ng/ml standard
⇩
Add blank, standard and samples to tubes (100 µl/tube)
⇩
Reconstitute the acridan/HRP conjugate mix (5.5 ml/vial)
⇩
Add conjugate mix to the tubes (0.5 ml/tube)
⇩
Incubate tubes at room temp for 45 min
⇩
Measure luminescence

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 5 — Store at -20°C
- Dog S100A8 stock — Store at -20°C
- Diluent: CSD50-1, 2 x 50 ml
- Background reducer: BR9-1, 9 ml
- Trigger solution: TS12-1, 12 ml
- 15 ml centrifuge tubes, 2

Materials required but not provided:

- VetBio-1 luminometer
- Precision pipettors and tips
- 12 x 75 mm borosilicate tubes[2]
- 12 x 75 mm test tube racks

STORAGE

Store the conjugate and S100A8 stock vials at or below -20°C. The remainder of the kit should be stored at 2-8°C.

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 before use.

LUMINOMETER SETUP

1. Turn the VetBio-1 luminometer on.
2. Place the tubing from injector 1 into the tube containing background reducer. Tube holders are positioned adjacent to the injectors.
3. Place the tube from injector 2 into the tube containing trigger solution.
4. From the Protocol manager on the keypad select “Prime & Wash”, "Prime", then “Start”.
5. Open the luminometer drawer and insert an empty 12 x 75 mm tube.
6. Close the drawer and click “Start” again.
7. Wait for priming to complete, open the drawer and discard the tube.
8. From the Protocol manager select “Measure” followed by “Dog S100A8”.
9. Select “Start.

[1] SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.
[2] Only cylindrical 12 x 75 mm borosilicate glass tubes can be used. Do not use polystyrene or polycarbonate tubes.

10. The experiment setup screen will be displayed, allowing you to enter experiment name, comments, and the number of samples. After entering the information select "Start".
11. Enter the sample IDs and dilution factor(s), if different than the default dilution factor.
12. The luminometer is now ready for use.
13. Press "Start" when you are ready to measure luminescence.

REPLICATES

The VetBio-1 luminometer protocols are configured to run singlets of the blank, standard and samples. At the discretion of the user it can be configured to run replicates.

1. From the protocol manager select "Measure" and the program you would like to modify.
2. Select "New" and "Copy Protocol".
3. Increase the Replicates as desired.
4. Create a new protocol name.
5. Select "Protocols" and save the new protocol.

SAMPLE PREP

This assay was designed for measurement of S100A8 in serum. In studies at Veterinary Biomarkers, Inc., we found S100A8 levels in serum ranging from 0 to 30 ng/ml. Testing at a dilution of 20-fold appeared optimal. A 20-fold dilution can be obtained by mixing 25 µl of serum with 475 µl of CSD50-1 diluent. Avoid testing highly hemolyzed samples because false low values may be obtained.

STANDARD PREP

1. The S100A8 stock is provided in liquid form. It should be stored at or below -20°C.
2. Prepare the 10 ng/ml S100A8 standard by mixing 10.0 µl of the stock with 0.99 ml of CSD50-1 diluent.
3. Use the standard within 30 min of reconstitution.

CONJUGATE PREP

1. The acridan and HRP conjugate mix should be prepared just before use (step 5 in the Procedure section).
2. Tap the vial to ensure that the contents are at the bottom of the vial before carefully removing the stopper.
3. Add 5.5 ml of diluent CSD50-1 to the vial. Insert the stopper and mix gently by inversion several times.
4. Each vial of reconstituted conjugate mix provides enough reagent to measure 0 and 10 ng/ml standards and up to eight samples.[3]

PROCEDURE

1. Determine the number of 12 x 75 mm borosilicate glass tubes required for the assay. Ensure that all tubes fit easily in the VetBio-1 sample holder.
2. Pipet 100 µl of diluent into assay tube one. This serves as the zero standard.
3. Pipet 100 µl of the 10 ng/ml S100A8 standard into tube two.
4. Pipet 100 µl aliquots of the diluted samples into tubes 3, 4, 5… as defined by your assay format.
5. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available.
6. Incubate the mixtures at room temperature.
7. After 45 minutes insert tube 1 into the sample holder of the luminometer and close the drawer. The luminometer automatically injects background reducer and trigger solution, then measures luminescence (RLU/s).
8. Once the RLU/s value is recorded on the screen open the drawer and discard the tube.
9. Determine luminescence for the remaining tubes.
10. S100A8 concentrations are automatically calculated.
11. After measurement of the last sample, select "End".
12. Results will be saved but may be exported as Excel or pdf files via a USB stick.

LUMINOMETER MAINTENANCE

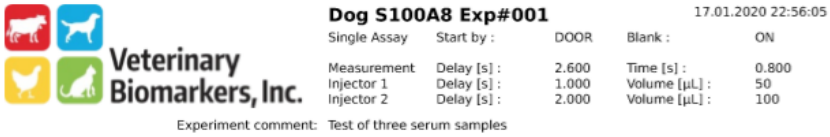
The luminometer injectors must be cleaned with distilled or deionized water at the end of each day the instrument is used to avoid clogging of the injector ports.

1. From the Protocol manager screen select "Prime & Wash".
2. Select "Backprime" to return unused reagents to the respective tubes.
3. If future use of the reagents is intended store the sealed tubes in a refrigerator.
4. Place the tubing from injectors 1 and 2 into separate 15 ml centrifuge tubes containing distilled or deionized water.
5. Select "Wash" from the "Prime & Wash" screen. Press "Start", insert an empty tube into the luminometer, close the drawer and press "Start".
6. Discard the tube.
7. Leave the injector tubing immersed in water.
8. Switch the luminometer off. It should not be left on.

[3] If using multiple vials of conjugates to measure more than eight samples, combine the reconstituted contents of all vials and mix briefly before dispensing 0.5 ml aliquots into the reaction tubes.

TYPICAL DATA

The figure below shows results from an assay in which the zero standard (blank), 10 ng/ml standard, and three serum samples were tested as singlets at a 20-fold dilution.



Dog S100A8 Exp#001 17.01.2020 22:56:05

Single Assay	Start by :	DOOR	Blank :	ON
Measurement	Delay [s] :	2.600	Time [s] :	0.800
Injector 1	Delay [s] :	1.000	Volume [µL] :	50
Injector 2	Delay [s] :	2.000	Volume [µL] :	100

Experiment comment: Test of three serum samples

Sample	Dilution	Rep	RLU/s	Conc [ng/mL]
Blank			990	0
Standard			278,035	10
152	20		18,825	12.875
154	20		26,918	18.717
156	20		10,372	6.7733

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