



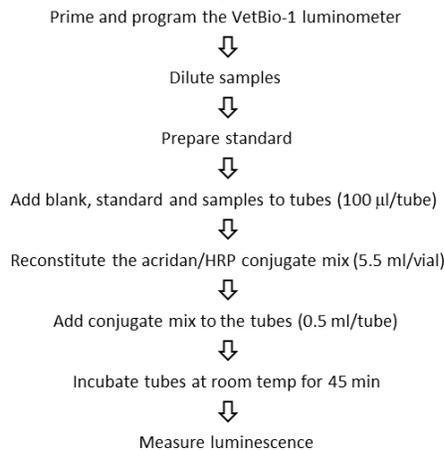
INTRODUCTION

Lactoferrin (LF) is a non-heme iron binding glycoprotein found in milk, feces and blood. As a component of host defense, it has antimicrobial and anti-inflammatory activity. In humans, it is used as a biomarker of intestinal inflammation. The utility of LF as a biomarker in dogs is under active investigation.

PRINCIPLE OF THE ASSAY

The dog LF assay uses SPARCL™¹ technology. Two LF antibody conjugates are used; one to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugates bind to LF 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 LF 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 LF is determined from the ratio of sample luminescence to that of the 20 ng/ml standard.



MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 5 **Store at -20°C**
- Dog LF stock, 100 µl **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 tubes²
- 12 x 75 mm test tube racks

STORAGE

Store the conjugate and LF 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 LF".
9. Select "Start".
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".

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.

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 LF in serum and fecal extracts. In studies at Veterinary Biomarkers, Inc., we found LF levels in serum ranging from 0 to 300 ng/ml. We suggest that serum be tested at a 20-fold dilution, obtained by mixing 20 μ l of serum with 380 μ l of CSD50-1 diluent. Fecal extracts should be tested at dilutions of 25-fold or greater.

STANDARD PREP

1. The LF stock is provided as a 100-fold concentrate.
2. Aliquot 0.99 ml of CSD50-1 diluent to a microcentrifuge tube. Add 10.0 μ l of the 100-fold stock and mix. This provides the 20 ng/ml standard.
3. Use the standard within 30 min of preparation.

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 20 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 μ l of diluent into assay tube one. This serves as the zero standard.
3. Pipet 100 μ l of the 20 ng/ml LF standard into tube two.
4. Pipet 100 μ l 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. LF 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.

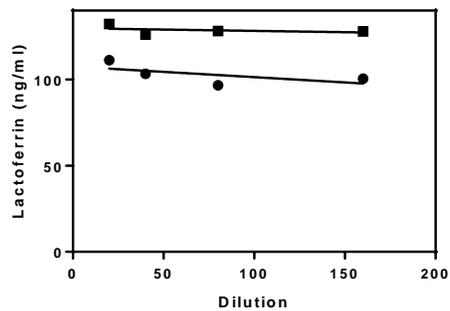
³ If using multiple vials of conjugates to measure more than eight samples, combine the reconstituted contents of all vials and mix briefly before dispensing 0.5 ml aliquots into the reaction tubes.

ASSAY PERFORMANCE

Typical data: The table below shows results from an assay in which the zero standard (blank), 20 ng/ml standard, and ten serum samples were tested in duplicate.

Veterinary Biomarkers, Inc.		Dog Lactoferrin Exp#001		15.01.2020 23:27:30	
		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: Serum samples in duplicate					
Sample	Dilution	Rep	RLU/s	Conc [ng/mL]	
Blank		1	4,970	0	
		2	4,905	0	
		Avg	4,938	0	
Standard		1	231,984	20.057	
		2	230,698	19.943	
		Avg	231,341	20	
26	20	1	116,795	197.63	
		2	126,795	215.29	
		Avg	121,795	206.46	
46	40	1	338,565	1,178.9	
		2	335,670	1,168.6	
		Avg	337,118	1,173.8	
65	20	1	32,325	48.387	
		2	32,152	48.082	
		Avg	32,239	48.235	
83	20	1	76,774	126.92	
		2	76,470	126.38	
		Avg	76,622	126.65	
102	20	1	93,248	156.02	
		2	95,189	159.45	
		Avg	94,218	157.74	
119	40	1	36,602	111.89	
		2	36,434	111.29	
		Avg	36,518	111.59	
121	20	1	234,409	405.42	
		2	236,216	408.61	
		Avg	235,312	407.02	
152	20	1	154,595	264.41	
		2	154,065	263.47	
		Avg	154,330	263.94	
168	20	1	126,720	215.16	
		2	125,881	213.68	
		Avg	126,301	214.42	
175	20	1	27,734	40.276	
		2	26,876	38.761	
		Avg	27,305	39.518	

Linearity: To assess the linearity of the assay, serum samples containing LF at concentrations of 103 and 129 ng/ml were serially diluted with diluent CSD50-1 to produce values within the dynamic range of the assay.



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