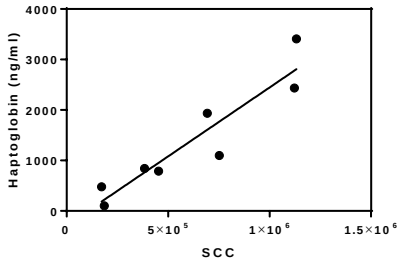
COW HAPTOGLOBIN VETBIO-1 ASSAY HAPT-COW-VB

INTRODUCTION

Haptoglobin is an acute phase protein. Haptoglobin expressed in liver circulates in blood, that expressed in mammary epithelial cells is found in milk. Levels in milk increase during inflammatory disease and bacterial infections. It is a potential biomarker for mastitis. Studies at Veterinary Biomarkers Inc. indicate that milk haptoglobin levels correlate with somatic cell count (SCC).



The cow haptoglobin assay uses SPARCL™[1] technology. It uses two haptoglobin antibodies. One is conjugated to horseradish peroxidase (HRP), the other to acridan; a chemiluminescent substrate. When HRP and acridan conjugates bind to haptoglobin 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 haptoglobin concentration.

Diluted milk samples and standards 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 a background reducer, that eliminates nonspecific luminescence, followed by trigger-solution containing hydrogen peroxide. The concentration of haptoglobin is determined from the ratio of sample luminescence to the that of the 100 ng/ml standard.

Prime and program the VetBio-1 luminometer

⇩

Dilute samples

⇩

Prepare 100 ng/ml standard

⇩

Add blank, standard and samples to tubes (100 µl/tube)

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Reconstitute the acridan/HRP conjugate mix (5.5 ml/vial)

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Add conjugate mix to the tubes (0.5 ml/tube)

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Incubate tubes at room temp for 45 min

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Measure luminescence

STORAGE

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

KIT COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 5 vials Store at -20°C
- Haptoglobin standard, 5 vials Store at -20°C
- Diluent: CSD50-1, 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

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.

[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.

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 “Cow haptoglobin (milk)” if measuring milk samples or “Cow haptoglobin” if measuring serum samples.
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”.
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

Milk: This assay was designed for measurement of haptoglobin in milk. In milk with somatic cell counts in the range of 0.06 to 1.1 x 10^6 we found haptoglobin levels ranging from 100 to 4000 ng/ml. We suggest testing milk of normal appearance at a dilution of 50-fold, but optimum dilutions must be determined empirically. A 50-fold dilution can be obtained by mixing 10.0 µl of milk with 0.49 ml of CSD50-1 diluent. To avoid matrix effects do not test milk at dilutions less than 50-fold.

Serum: The assay can be used to measure haptoglobin in serum and plasma but because of the wide range of possible levels, optimal dilution factors must be determined empirically. In serum from healthy cows we found levels of ~ 0.1 mg/ml. In serum from sick cows, levels >2 mg/ml were found, the latter necessitating a dilution of 400,000-fold to obtain luminescence values within the standard range.

STANDARD PREP

The standard is provided lyophilized. Reconstitute with the volume of CSD50-1 diluent indicated on the vial label to obtain the 100 ng/ml standard.

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 100 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 100 ng/ml haptoglobin 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 50 µl of background reducer and 100 µl of 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. haptoglobin 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.

[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. Larger volumes of background reducer and trigger solution must also be used.

LUMINOMETER MAINTENANCE

The luminometer injectors must be cleaned with distilled or deionized water at the end of each day of use 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 when not in use.

ASSAY PERFORMANCE

Typical data: The table below shows results from an assay in which the zero standard (blank), 100 ng/ml standard, and eight milk samples were tested as singlets.

Veterinary Biomarkers, Inc.

Cow Haptoglobin (milk) Exp#001 06.02.2020 00:06:12

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

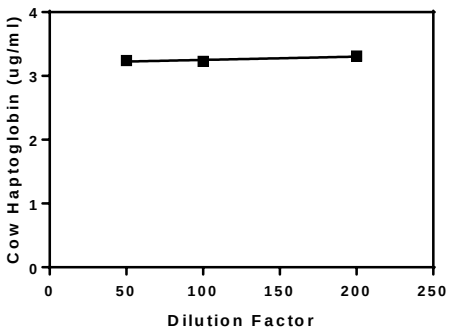
Experiment comment: Test of milk with a range of SCC

Sample	Dilution	Rep	RLU/s	Conc [ng/mL]
Blank			715	0
Standard			154,298	100
391, 1	100		14,056	868.67
391, 2	100		20,685	1,300.3
391, 3	100		52,600	3,378.3
391, 4	100		88,981	5,747.2
394, 1	100		2,630	124.69
394, 2	100		22,978	1,449.5
394, 3	100		27,371	1,735.6
394, 4	100		60,638	3,901.6

Reproducibility: Three samples with different SCC were tested in triplicate in three separate assays. Levels of haptoglobin are reported as µg/ml (mean ± SD).

SCC (cells/ml)	Assay 1	Assay 2	Assay 3
453,000	0.68±0.02	0.68±0.02	0.89±0.06
693,000	2.00±0.11	1.99±0.03	2.56±0.16
1,132,000	3.49±0.07	3.44±0.05	4.78±0.18

Linearity: To assess the linearity of the assay, a milk sample containing haptoglobin at a concentration of 3.26 µg/ml was serially diluted with diluent CSD50-1 to produce values within the dynamic range of the assay.



Rev 041120

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