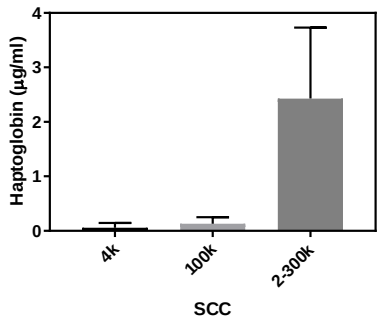
Cow Haptoglobin Sirius SPARCL Kit, Cat. No. HAPT-SPL-11

INTRODUCTION

Haptoglobin is a positive acute phase protein. Levels increase in serum because of injury and infection; they can be used to diagnose and monitor diseaseHaptoglobin is also elevated in milk due to mastitis. As shown in the figure below, levels increase significantly at somatic cell counts of 200,000 or greater.



The cow haptoglobin Sirius SPARCL™[1] assay allows rapid measurement of haptoglobin using a Berthold Sirius-L single tube luminometer.

PRINCIPLE OF THE ASSAY

The assay uses two haptoglobin-specific antibodies. One conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies 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 serum samples (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 Sirius-L luminometer. Luminescence is measured after automatic injection of background reducer, that eliminates nonspecific luminescence, followed by trigger-solution containing hydrogen peroxide. The concentration of haptoglobin is calculated from the ratio of sample luminescence to the luminescence of a haptoglobin standard.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 1 vial Store at -20°C
- Cow haptoglobin standard, 1 vial Store at -20°C
- Diluent; CSD50-1, 50 ml
- Background reducer; BR4-1, 4 ml
- Trigger solution; TS4-1, 4 ml
- 15 ml centrifuge tubes, 4

Materials required but not provided:

- Sirius-L luminometer with two injectors and PC
- Precision pipettors and tips
- Test tubes (borosilicate, 12 x 75 mm)
- 12 x 75 mm test tube rack
- Excel software

STORAGE

Store the conjugate and standard 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 removed from the refrigerator or freezer and allowed to reach room temperature (25°C) before use.
3. Follow the sequence of events below when running the assay.

Prime and program the Sirius-L luminometer
⇩
Prepare standards (0 & 50 ng/ml) and diluted samples
⇩
Add standards and samples to the 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

LUMINOMETER SETUP

1. Switch the Sirius-L luminometer on and allow it to warm up for at least 15 minutes.
2. Place the tubing from injector one into a 5 ml centrifuge tube containing background reducer.
3. Place the tube from injector two into a 5 ml centrifuge tube containing trigger solution.
4. Open the Sirius-L software on the computer. The Protocol Manager screen will load.
5. Click on “Priming Injectors” and program the luminometer to prime injector-1 with 3 x 0.5 ml of background reducer and injector-2 with 3 x 0.5 ml of trigger solution. Make sure to select “to tube” as the destination.
6. Open the luminometer drawer and insert an empty 12 x 75 mm test tube.
7. Close the drawer and click “Prime”
8. Wait for priming to complete then open the drawer and discard the tube.
9. Highlight the Quick Measurement program in the Protocol Manager window and click “Edit”. Enter the following parameters:

[1] SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.

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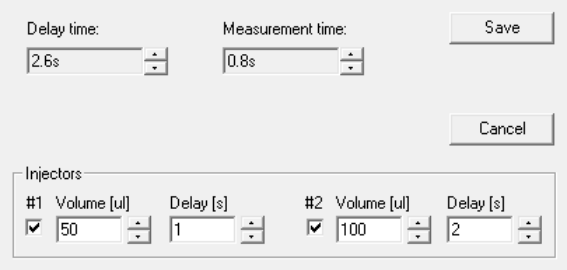


10. Click “Save”.
11. Highlight Quick Measurement and click “Run”.
12. The luminometer is now ready for use.

ASSAY FORMAT

1. Download the Excel spreadsheet from the Life Diagnostics website: https://lifediagnostics.com/cow-haptoglobin-spreadsheet/
2. The first two rows/tubes are reserved for the zero and 50 ng/ml standards.
3. Enter your sample identities and dilution factor(s) (we recommend 500-fold) into rows 3+ in the order that you intend to measure them.

SAMPLE PREPARATION

Serum/Plasma. Haptoglobin levels can range from 0.1 to >3 mg/ml depending on health status. We recommend that serum or plasma be tested initially at a dilution of 10,000-fold[2]:

1. For each sample dispense 495 µl of CSD50-1 diluent into two tubes.
2. Mix 5.0 µl of serum or plasma with 998 µl of diluent in the first tube to give a 100-fold dilution.
3. Mix 5.0 µl of the 100-fold diluted sample with 495 µl of diluent in the second tube to give a 10,000-fold dilution.

Milk. We found haptoglobin levels ranging from undetectable in milk from healthy cows to 4 µg/ml in milk with cows with somatic cell counts of 200-300,000. We recommend that milk be tested initially at a dilution of 100-fold:

1. For each sample dispense 495 µl of CSD50-1 diluent into a test tube.
2. Add 5.0 µl of milk and mix to give a 100-fold dilution.

STANDARD PREPARATION

1. Reconstitute the 50 ng/ml haptoglobin standard with the volume of CSD50-1 diluent indicated on the vial label.
2. 100 ul is pipetted into tube 2 as described in the Procedure section.

CONJUGATE MIX PREPARATION

1. The acridan and HRP conjugate mix should be prepared just before use (step 6 in the Procedure section).
2. Add 5.5 ml of diluent CSD50-1 to the vial that contains the freeze-dried conjugates and mix gently by inversion 20x.
3. Each vial of reconstituted conjugate mix provides enough reagent to measure 0 and 50 ng/ml standards and up to eight samples.[3]

PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with background reducer and trigger solution and that the SPARCL program is loaded (refer to the Luminometer Setup section).
2. Prior to starting the assay ensure that the 12 x 75 mm borosilicate test tubes fit easily in the luminometer cuvette holder.
3. Pipet 100 µl of diluent into tube one. This serves as the zero standard.
4. Pipet 100 µl of the reconstituted 50 ng/ml haptoglobin standard into tube two.
5. Pipet 100 µl aliquots of the diluted samples into tubes 3, 4, 5… as defined by your assay format.
6. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available.
7. Incubate the mixtures at room temperature.
8. 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).
9. Once the RLU/s value is recorded on the screen open the drawer and discard the tube.
10. Determine luminescence for the remaining tubes.

CALCULATION OF RESULTS

1. Cut and paste the RLU/s values determined by the luminometer into the RLU/s column of the downloaded Excel spreadsheet.
2. haptoglobin values (ug/ml) are automatically calculated.

TYPICAL RESULTS

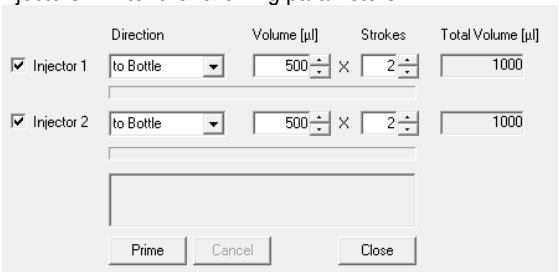
Typical results for the 0 and 50 ng/ml standards and six cow serum samples are shown below.

Cow Haptoglobin SIRIUS Worksheet						
#	Sample ID	Comments	Dilution	RLU/s	Zero subtracted RLU/s	ug/ml
1	0 ng/ml Hapt			6758	0	
2	50 ng/ml Hapt			143164	136406	
3	H1	Healthy	20	21273	14515	0.11
4	H2	Healthy	20	20316	13558	0.10
5	M1	Mastitis	1,000	90621	83863	30.74
6	M2	Mastitis	1,000	92542	85784	31.44
7	S1	Sick/non-mastitis	100,000	90391	83633	3065.59
8	S2	Sick/non-mastitis	100,000	64254	57496	2107.53

LUMINOMETER MAINTENANCE

The luminometer injectors should be cleaned with distilled or deionized water at the end of each day of use.

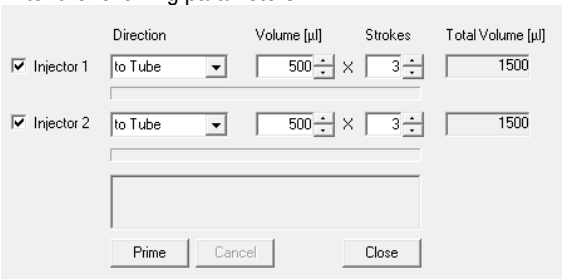
1. Exit the Quick Measurement screen and click on “Priming Injectors”. Enter the following parameters:



[2] Because of the homogeneous nature of the assay it is susceptible to a prozone effect. Samples with high haptoglobin levels may give false low values if tested at low dilutions. We therefore recommend testing several dilutions per sample.

[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.

2. Click “Prime”. The contents of the injectors will be returned to the respective tubes. If future use is intended store the sealed tubes in a refrigerator.
3. Place the tubing from injectors one and two into separate tubes containing distilled or deionized water.
4. Enter the following parameters:



5. Insert an empty 12 x 75 mm test tube into the luminometer. Close the drawer and click “Prime”.
6. After priming is complete discard the 12 x 75 mm tube and its contents.

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