

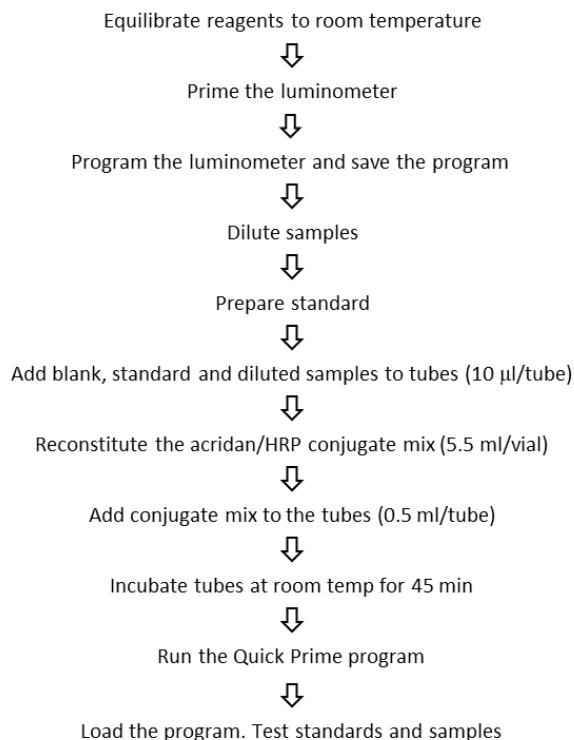


INTRODUCTION

Haptoglobin is an acute phase protein. It is expressed in liver and circulates in blood. Serum levels increase during bacterial infections and inflammatory diseases. Increased fecal levels are associated with inflammatory bowel disease.

The dog haptoglobin assay uses SPARCL™¹ technology. Two haptoglobin antibody conjugates are used. One 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.

In practice, a blank, a standard, and diluted samples (10 µl) are pipetted into test tubes and mixed with 0.5 ml of combined acridan and HRP conjugates. After 45 minutes, the tubes are sequentially inserted into the VetBio-1 luminometer. Luminescence is measured after injection of background reducer, followed by hydrogen peroxide containing trigger-solution. The concentration of haptoglobin is determined from the ratio of blank-subtracted sample luminescence to the that of the standard.



STORAGE

Store the conjugate and haptoglobin standard vials at or below -20°C. The remainder of the kit should be stored at 4°C.

MATERIALS AND 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 tubes²
- 12 x 75 mm test tube racks
- De-ionized water
- 15-ml centrifuge tubes & rack
- 10-ml pipets

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 new experiment" followed by "Dog Haptoglobin (Serum)" or "Dog Haptoglobin (Feces)".
9. Select "Start".
10. The experiment setup screen will be displayed. Enter the number of samples you intend to measure, then press "OK". Please note that the number of samples does not include the two tubes used for the blank and standard.
11. Enter the sample IDs and dilution factor(s), if different than the default dilution. You can also enter experimental comments.
12. After entering information, press save. The experiment will be saved as a "prepared experiment".

SAMPLE PREPARATION

Serum and Plasma

We recommend testing at a dilution of 5000-fold.

1. For each sample to be tested pipet 10 ml of deionized water into a test tube.
2. Add 2.0 ul of sample and mix. This provides a 5000-fold dilution.
3. Use 10.0 ul as described in the procedure section.

Feces

We recommend that feces be extracted and diluted as follows.

1. Accurately weigh approximately 100 mg of feces into a tared 1.5 ml microcentrifuge tube.
2. Add 9 volumes of CSD50-1 or 10 mM Tris, 150 mM NaCl, 1 mM EDTA pH 7.5 (i.e., 0.9 ml to 100 mg of feces).
3. Vortex several times over a 30-minute period to prepare a suspension that is as homogeneous as possible.
4. Centrifuge in a microcentrifuge (5 minutes at 15,000 rpm).
5. Save the supernatant. This represents a 10-fold "dilution" of the fecal sample. Samples may be stored frozen at or below -20°C.
6. For each sample to be tested mix 10 µl of the 10-fold dilution with 0.49 ml of water to obtain a 500-fold dilution.
7. Test 10.0 ul as described in the procedure section.

STANDARD PREPARATION

1. The haptoglobin standard is provided in lyophilized form.
2. Add the volume of CSD50-1 diluent indicated on the vial label and mix. Use a vortex mixer if available. This provides a 400 ng/ml standard.
3. Pipet 10.0 ul into tube 2 as described in procedure section.

Use the standard within 30 min of reconstitution.

CONJUGATE PREPARATION

1. The acridan and HRP conjugate mixture should be prepared just before use.
2. Tap the vial to ensure that the contents are at the bottom of the vial before slowly removing the stopper (it is under vacuum).
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 the blank, standard, and up to eight samples.³

PROCEDURE

STEP 1

1. Determine the number of 12 x 75 mm borosilicate glass tubes required (one each for the blank, standard, and the samples). Ensure that all the tubes you intend to use fit easily in the VetBio-1 sample holder before beginning the test.
2. Pipet 10 µl of diluent into tube 1. This serves as the zero standard or blank.
3. Pipet 10 µl of the 400 ng/ml haptoglobin standard into tube 2.
4. Pipet 10 µl of the diluted samples into tubes 3, 4, 5...
5. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available. Avoid creating bubbles.
6. Incubate the mixtures at room temperature for 45 minutes (incubations as short as 15-minutes can be used if testing eight or fewer samples).

STEP 2

1. Two to three minutes before the end of the incubation step, insert an empty tube into the sample holder. Select "QuickPrime" from the Protocol manager, close the drawer and press start. Discard the tube after completion. This step ensures that the injectors are fully loaded.
2. Recall your saved experiment and press start.
3. After the end of the incubation, insert tube 1 (Blank) into the sample holder of the luminometer and close the drawer. When the luminescence value is recorded on the screen open the drawer and remove the tube.
4. Similarly measure luminescence of the standard (tube 2), followed by the samples (tubes 3, 4, 5...).
5. Haptoglobin concentrations of the samples are automatically calculated.

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.

- After measurement of the last sample, select "End".
- Results will be saved and can be recalled. They may be exported as Excel or pdf files via a USB stick.

RESULTS

If RLU/s values for diluted samples are between the blank and standard, assume that the calculated values are correct. If RLU/s values significantly exceed that of the standard, haptoglobin concentrations may be underestimated. If an accurate measurement is required, such samples should be retested at a higher dilution.

LUMINOMETER MAINTENANCE

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

- From the Protocol manager screen select "Prime & Wash".
- Select "Backprime" to return unused reagents to the respective tubes.
- Cap the background reducer and trigger solution tubes and store them at 4°C.
- Wipe the tubing from injectors 1 and 2 and place them in separate 15 ml centrifuge tubes containing distilled or deionized water.
- Select "Wash" from the "Prime & Wash" screen. Press "Start", insert an empty tube into the luminometer, close the drawer and press "Start".
- Discard the tube.
- Insert an empty tube and repeat steps 5 and 6.
- Leave the injector tubing immersed in water.
- Switch the luminometer off. It should not be left powered-up overnight.

REPLICATES


Replicate measurements of the blank/standard and samples can be measured. This requires minor editing of the program.

- In Protocol manager, select "Measure new experiment" and the program you would like to edit.
- Press "Edit".
- You can change the sample dilution factor, the number of replicates for the blank and standard, and the number of replicates for samples (leave the standard concentration as 400 ng/ml).
- Press Start, enter the number of samples, and save the experiment.


Please note that these settings will be saved for future runs unless re-edited.

ASSAY PERFORMANCE

Typical data: The tables below show results from assays in which the zero standard (blank), 400 ng/ml standard, and eight serum, and eight fecal samples were tested.

		Dog Haptoglobin (Serum) Exp#011 10/08/2021 11:21:03			
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	

Sample	Dilution	Rep	RLU/s	Conc [mg/mL]
Blank			6,561	0
Standard			412,448	4e-4
9	5000		345,951	1.6723
10	5000		7,069	0.0025007
11	5000		686,948	3.3526
12	5000		79,848	0.36112
13	5000		15,846	0.045752
14	5000		19,915	0.0658
15	5000		824,465	4.0302
16	5000		1,106,282	5.4189

		Dog Haptoglobin (Feces) Exp#012 10/08/2021 11:29:50			
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	

Sample	Dilution	Rep	RLU/s	Conc [μg/mL]
Blank			6,058	0
Standard			387,494	0.4
9	500		184,128	93.368
10	500		13,821	4.0708
11	500		128,545	64.224
12	500		296,890	152.49
13	500		9,769	1.9459
14	500		12,356	3.3026
15	500		74,254	35.758
16	500		657,092	341.36