CAT HAPTOGLOBIN VETBIO-1 ASSAY HAPT-CAT-VB

INTRODUCTION

Haptoglobin is a positive acute phase protein that is expressed in liver and circulates in blood. Levels increase during disease and inflammation (refs. 1-2). It can be used as a biomarker to monitor disease progression.

The cat 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 serum or plasma 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 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)

⇩

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

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Incubate tubes at room temp for 45 min

⇩

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 stock, Store at or below -20°C
- Diluent: CSD50-1, 2x50 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.

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 “Cat haptoglobin”.
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

We found that haptoglobin levels ranged from 0.1 to 8 mg/ml. A dilution of 50,000-fold worked well for most samples. A 50,000-fold dilution can be obtained as follows.

1. For each sample to be tested pipette 0.996 ml and 0.995 ml of diluent into separate tubes.
2. Prepare a 250-fold dilution by mixing 4 µl of sample with 0.996 ml of diluent.
3. Prepare a 50,000-fold dilution by mixing 5 µl of the 250-fold diluted sample with 0.995 ml of diluent in the second tube.

STANDARD PREP

Prepare the 100 ng/ml standard by mixing 5 µl of the haptoglobin stock with 0.495 ml of diluent.

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.

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.

[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.

ASSAY PERFORMANCE

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

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Cat Haptoglobin Exp#002 10.04.2020 23:04:30

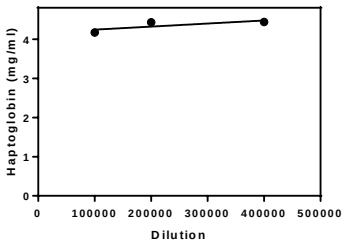
Single Assay	Start by :	DOOR	Blank :	ON	
Measurement	Delay [s] :	2.600	Time [s] :	0.800	
Injector 1	Delay [s] :	1.000	Volume [µL] :	50	
Injector 2	Delay [s] :	2.000	Volume [µL] :	100	

Sample	Dilution	Rep	RLU/s	Conc [mg/mL]
Blank			4,074	0
Standard			159,039	1e-4
7	50000		142,569	4.4686
14	50000		54,396	1.6237
22	50000		23,458	0.62542
23	50000		16,251	0.39291
26	50000		9,608	0.17855

Reproducibility: Five serum samples were tested in triplicate in three separate assays. Intra-assay and inter-assay variability are listed below.

	Intra-assay			Inter-assay		
Sample	mg/ml	SD	CV	mg/ml	SD	CV
7	4.74	0.04	0.7	4.49	0.39	8.7
14	1.58	0.05	3.4	1.50	0.13	8.4
22	0.65	0.01	1.2	0.61	0.03	5.6
23	0.54	0.01	1.9	0.50	0.03	6.4
26	0.22	0.01	3.6	0.21	0.01	5.0

Linearity: To assess the linearity of the assay, a serum sample containing haptoglobin at a concentration of 4.3 mg/ml was serially diluted with diluent CSD50-1 to produce values within the dynamic range of the assay.



REFERENCES

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2. Stiller J. et al. Validation of an enzyme-linked immunosorbent assay for measurement of feline haptoglobin. Journal of Veterinary Diagnostic Investigation. 28(3):235-243 (2016)

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