INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in the serum of most mammals during injury, infection and disease. In cats, it has been reported that serum haptoglobin increases up to ten-fold during the acute phase response (ref 1).

PRINCIPLE OF THE ASSAY

The cat haptoglobin SPARCL™1 (Spatial Proximity Analyte Reagent Capture Luminescence, ref 2) assay uses two cat haptoglobin-specific antibodies. One is conjugated to horseradish peroxidase (HRP), the other is conjugated to acridan, a chemiluminescent substrate. When the HRP and acridan conjugated antibodies bind to haptoglobin they are brought into close proximity. With the addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies distant from HRP produce no signal. This principle allows the development of a homogeneous assay that allows rapid measurement of haptoglobin concentrations.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well SPARCL™ plate provided with the kit2. After incubation for 30 minutes on a shaker at 25°C and 150 rpm, the plate is placed into a luminometer. Trigger solution containing hydrogen peroxide is injected into each well and luminescence is immediately measured. The concentration of haptoglobin is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- Anti-haptoglobin HRP conjugate  Store ≤ -70°C
- Anti-haptoglobin acridan conjugate  Store ≤ -70°C
- Haptoglobin stock (3 vials)  Store ≤ -70°C
- Diluent; CSD50-1, 2 x 50 ml
- Trigger solution; TS7-1, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP conjugate, acridan conjugate and haptoglobin stock at -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described above.

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 (25°C) before use.
3. It is important that standards and samples be added to the SPARCL™ plate quickly. If testing large numbers of samples, rather than pipetting standards and samples directly into the white SPARCL™ plate using a single channel pipettor, we recommend the following. First, pipette an excess volume of standards and samples into appropriate wells of the clear 96-well plate. Then use an 8- or 12-channel multipipettor to quickly and efficiently transfer 50 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate hold a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.
   - Prime and program the Luminometer
   - Prepare standards and diluted samples
   - Prepare HRP + Acridan conjugate mix
   - Add HRP + Acridan conjugate mix to the wells (50 µl)
   - Add standards and samples to the wells (50 µl)
   - Incubate plate at 150 rpm/25°C for 30 min
   - Measure luminescence after injection of Trigger (37.5 µl)

STANDARD PREPARATION

The cat haptoglobin stock is comprised of lyophilized cat haptoglobin in a carrier protein matrix. The haptoglobin content was determined by reference to purified cat haptoglobin prepared at Life Diagnostics, Inc.

1. Reconstitute the lyophilized cat haptoglobin stock with diluent as described on the vial label. Mix gently until dissolved. The concentration of haptoglobin in the reconstituted stock is indicated on the label.
2. Label 8 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the haptoglobin stock vial label. Then add the indicated volume of haptoglobin stock and mix gently. This provides the 50 ng/ml standard.
4. Into the tube labeled 25 ng/ml, pipette the volume of diluent detailed on the haptoglobin stock vial label. Then add the indicated volume of haptoglobin stock and mix gently. This provides the 50 ng/ml standard.
5. Into the tube labeled 12.5 ng/ml, pipette the volume of diluent detailed on the haptoglobin stock vial label. Then add the indicated volume of haptoglobin stock and mix gently. This provides the 50 ng/ml standard.
6. Similarly prepare the remaining standards by serial dilution.

Please Note: Use the standards within one hour of preparation.

---

1 The SPARCL technology was developed by Lumigen Corp.
2 The plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.
SAMPLE PREPARATION
Serum or heparinized plasma should be prepared as quickly as possible after blood collection. If samples cannot be assayed immediately they should be frozen at or below –20°C. Avoid repeated freeze-thaws.

The cat haptoglobin SPARCL assay uses a homogeneous format and is therefore susceptible to a prozone or “hook effect” at high haptoglobin concentrations. We found that at a dilution of 20,000-fold all plasma samples gave values within range of the standard curve and a prozone effect could be avoided. However, optimal dilutions should be determined by the end user. In our studies, we found that plasma haptoglobin concentrations ranged from 80 to 600 μg/ml.

A dilution of 20,000-fold can be achieved as follows:
1. Dispense 198 μl and 398 μl of diluent into separate tubes.
2. Pipette and mix 2.0 μl of the serum sample into the tube containing 198 μl of diluent. This provides a 100-fold dilution.
3. Mix 2.0 μl of the 100-fold diluted sample with the 398 μl of diluent in the second tube. This provides a 20,000-fold dilution.

CONJUGATE MIX PREPARATION
Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. If necessary, after blood collection. If samples cannot be assayed immediately they should be frozen at or below –20°C. Avoid repeated freeze-thaws.

A typical standard curve with RLU versus log10 haptoglobin concentration is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve should be run with each experiment.

REFERENCES
1. Kajikawa T. et. al. Changes in concentrations of serum amyloid A protein, α1-acid glycoprotein, haptoglobin and C-reactive...
protein in feline sera due to induced inflammation and surgery. Veterinary Immunology and Immunopathology. 68:91-98 (1999)


Rev 021618
For technical assistance please email us at techsupport@lifediagnostics.com