

# HUMAN HAPTOGLOBIN SPARCL™ ASSAY

## Life Diagnostics, Inc., Catalog Number: HAPT-SP-20

### FOR RESEARCH USE ONLY

#### INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in the serum of most mammals during injury, infection and disease. In humans, serum haptoglobin increases up to 10-fold during the acute phase response (ref 1).

#### PRINCIPLE OF THE ASSAY

The human haptoglobin SPARCL™<sup>1</sup> (Spatial Proximity Analyte Reagent Capture Luminescence, ref 2) assay allows measurement of haptoglobin using a single 30-minute incubation that requires no wash steps. The assay uses two haptoglobin-specific antibodies. One is conjugated to horse radish peroxidase (HRP); the other is conjugated to acridan, a chemiluminescent substrate. When HRP and acridan conjugated haptoglobin 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 determination 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 kit<sup>2</sup>. 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 **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

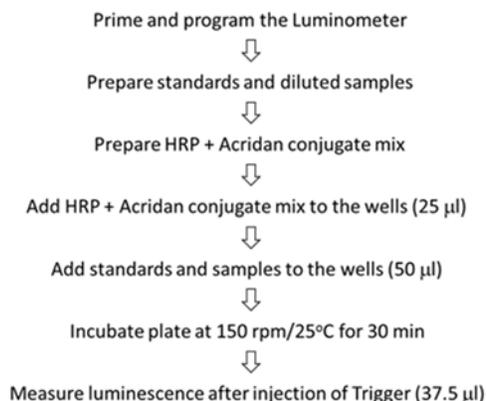
#### STORAGE

Store the HRP conjugate, acridan conjugate and haptoglobin stock at or below -70°C (they may be stored at -20°C for at least two weeks). 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 appropriately.

#### 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.



#### STANDARD PREPARATION

The human haptoglobin stock is comprised of purified human haptoglobin<sup>3</sup> diluted in a carrier protein matrix.

1. Thaw the haptoglobin stock shortly before use.
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. Dispense 150 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
5. Pipette 150 µl of the 50 ng/ml haptoglobin standard into the tube labeled 25 ng/ml and mix. This provides the 25 ng/ml haptoglobin standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

<sup>1</sup> The SPARCL technology was developed by Lumigen Corp.

<sup>2</sup> The plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

<sup>3</sup> Human haptoglobin (catalog no. H3536) from Sigma-Aldrich, St-Louis, MO, USA was used to prepare standards.

Use the standards within 30 minutes of preparation. Store unused haptoglobin stock at or below -70°C if future use is intended.

### SAMPLE PREPARATION

Because the human haptoglobin SPARCL™ assay uses a homogenous format, a prozone or hook effect can occur at high haptoglobin concentrations. We found that for most samples prozone and matrix effects could be eliminated if samples were tested at dilutions of 40,000-fold or greater. A 40,000-fold dilution can be achieved as follows.

1. Dispense 497.5 µl of diluent into two tubes.
2. Pipette 2.50 µl of serum or plasma into the first tube and mix. This provides a 200-fold dilution.
3. Pipette 2.50 µl of the 200-fold diluted sample into the second tube and mix. This provides a 40,000-fold dilution.

Use the samples within 30 minutes.

During validation of the assay we identified plasma and peritoneal fluid samples with haptoglobin concentrations ranging from 175 to 2270 µg/ml. Optimal dilutions of culture supernatants and tissue extracts must be determined empirically.

### CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. Prepare the mix shortly before use using the diluent provided with the kit.

### LUMINOMETER SETUP

1. The luminometer must be capable of injection and simultaneous measurement of luminescence without any delay.
2. Prime the luminometer injection port with 1 ml of trigger solution.
3. Place the injection needle into the injection port as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 µl of trigger solution per well and to measure from time zero for 1 second (50 x 0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as a 12 x 8-well strips, allowing use of fewer than 96-wells, make sure that the luminometer is programmed to inject trigger solution only into the wells being used.
7. We use a BMG LUMIstar Omega set at a gain of 3600. Optimal gain should be determined by the end user.
8. There are a number of manufacturers of luminometers that are equipped to run a SPARCL™ assay. Please contact Life Diagnostics or Lumigen ([www.lumigen.com](http://www.lumigen.com)) to discuss your luminometer.

### PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with trigger solution and that the injection needle is positioned in the injection port.
2. Secure the desired number of SPARCL™ 8-well strips in the holder. Immediately seal unused strips in the resealable bag with desiccant and antioxidant. Store unused strips at 2-8°C.
3. Aliquot 25.0 µl of conjugate mix into each well.
4. Dispense 50.0 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
5. Incubate on an orbital micro-plate shaker at 150 rpm 25°C for 30 minutes.

6. After the 30-minute incubation, place the plate in the luminometer and measure luminescence after injection of trigger solution (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

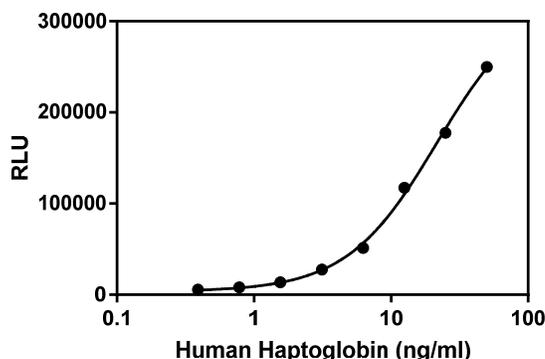
### CALCULATION OF RESULTS

1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells. We routinely use the sum of RLU values from a 100-980 ms data collection window.
2. Determine the sum of RLU values within the data collection window for the standards and samples.
3. Using graphing software, construct a standard curve by plotting the sum of the RLU values for the standards versus the log<sub>10</sub> of the haptoglobin concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of haptoglobin in the samples from the standard curve (remember to derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the concentration of haptoglobin in the original sample.
6. If the sum of the RLU values of diluted samples fall outside the standard curve, samples should be appropriately diluted and re-tested.

### TYPICAL STANDARD CURVE

A typical standard curve with sum of RLU versus log<sub>10</sub> 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.

Haptoglobin (ng/ml)	RLU
50	249920
25	177648
12.5	117331
6.25	51293
3.13	27548
1.56	13566
0.78	8136
0.39	5548



### REFERENCES

1. Heinrich PC, Castel JV and Andus T. Interleukin-6 and the acute phase response. *Biochem J.* 265:621-636 (1990)

2. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. *J Am Chem Soc.* 20;135(11):4191-4 (2013)

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This assay is for research purposes only.  
For technical assistance please email us at  
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