PIG MYOGLOBIN SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: MYO-SP-9

INTRODUCTION
Myoglobin is a 17 kDa heme protein that is expressed in both cardiac and skeletal muscle. After cardiac muscle injury myoglobin is one of the first biomarkers to increase above baseline values. In the absence of skeletal muscle injury, it can be used as a biomarker to assess cardiac damage. Similarly, in the absence of cardiac damage, myoglobin may be used as a biomarker of skeletal muscle injury.

PRINCIPLE OF THE ASSAY
The pig myoglobin SPARCL™ (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two different myoglobin-specific antibodies. An affinity purified polyclonal antibody is conjugated to horseradish peroxidase (HRP) and a myoglobin specific monoclonal antibody is conjugated to acridan, a chemiluminescent substrate. When HRP and acridan conjugated myoglobin antibodies bind to myoglobin they are brought into close proximity. With the addition of hydrogen peroxide, HRP catalyzes the oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies not bound to myoglobin antibodies bind to myoglobin they are brought into close proximity. The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well plate. Then use an 8- or 12-channel multipipettor to quickly transfer 50 µl aliquots to the appropriate wells of the clear 96-well plate. The wells of the clear plate have a maximum volume of 300 µl.

STANDARD PREPARATION
The pig myoglobin stock is comprised of pure pig myoglobin in a stabilizing buffer. Serum should be prepared as quickly as possible after blood collection and stored at 4°C (EDTA and citrate plasma cannot be used in this assay). All samples should be similarly processed i.e., storage times and temperatures should be the same. If serum samples cannot be tested immediately, they should be aliquoted and stored at or below -20°C. Avoid repeated freeze-thaws.

The levels of myoglobin depend on the degree of cardiac injury and the time after injury that blood is collected. Optimal dilution factors must therefore be determined empirically. However, all serum samples must be diluted at least 3-fold with the yellow sample diluent (YD25-1) in order to eliminate matrix effects. Do not substitute other dilution buffers.

In studies at Life Diagnostics, we found that pig serum collected at a local abattoir contained myoglobin levels of 945±522 ng/ml (mean±SD, n = 12, range = 205 - 1945 ng/ml).

STORAGE
Store the HRP and acridan conjugate at or below -70°C. The myoglobin stock should be stored at -20°C but must not be stored at lower temperatures. The remainder of the kit should be stored at 2 to 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.

GENERAL INSTRUCTIONS
1. Please take the time to completely read all of the 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 a large number 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 have a maximum volume of 300 µl.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- Anti-pig myoglobin HRP conjugate stock. Store ≤ -70°C
- Anti-pig myoglobin acridan conjugate stock. Store ≤ -70°C
- Pig myoglobin stock (1 vial). Store at -20°C
- Sample diluent (YD25-1)
- Conjugate diluent (CSD10-1)
- Trigger solution (TS11-1)
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:
- Precision pipettes, multi-pipettors and tips
- Polypropylene tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC graphing software

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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.
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 clear conjugate diluent (CSD10-1) for dilution.

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 at least 1 ml of trigger solution.
3. Place the injection needle into the injection port, (necessary for BMG luminometers).
4. Program the luminometer to inject 75 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) 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 (75 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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus Log\(_{10}\) myoglobin concentration.
3. Fit the data using a four parameter dose response model.
4. Derive the corresponding concentration of myoglobin in the samples from the standard curve.
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of myoglobin in the serum sample.

6. If the RLU values of diluted samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with RLU plotted on the Y-axis versus log\(_{10}\) myoglobin concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. A standard curve must be run with each experiment.

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<tr>
<th>Myoglobin (ng/ml)</th>
<th>RLU</th>
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<tr>
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Myoglobin (ng/ml) | RLU |
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REFERENCES


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