RAT MYOGLOBIN SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: MYO-SP-2

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 rat myoglobin SPARCL™[1] (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 oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies not bound to myoglobin and distant from HRP at a molecular level produce no signal. This principle allows the development of a homogeneous assay that allows rapid determination of myoglobin concentration.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well white SPARCL™ plate provided with the kit[2]. 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 myoglobin is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti- myoglobin HRP stock **Store ≤ -70°C**
- Anti- myoglobin acridan stock **Store ≤ -70°C**
- Mouse myoglobin stock **Store at -20°C**
- Sample diluent; YD25-1, 25 ml
- Conjugate diluent; CSD10-1, 10 ml
- Trigger solution; TS11-1, 11 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

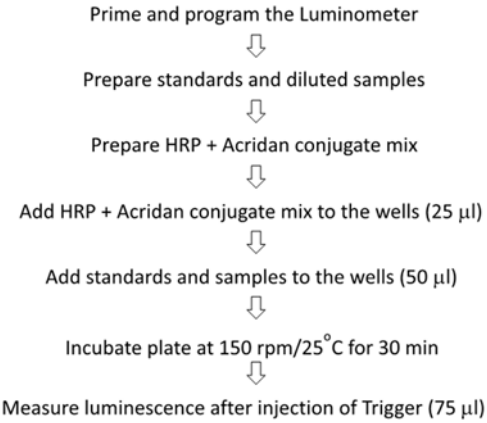
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP 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 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 many 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.
4. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The rat myoglobin stock is comprised of pure rat myoglobin at a concentration of 50 µg/ml in a stabilizing buffer.

1. Label 8 polypropylene tubes as 300, 150, 75, 37.5, 18.75, 9.38, 4.69 and 2.34 ng/ml.
2. Into the tube labeled 300 ng/ml, pipette 497.0 µl of YD25-1 diluent (yellow). Then add the 3.0 µl of 50.0 µg/ml myoglobin stock and mix gently. This provides the 300 ng/ml standard.
3. Dispense 250 µl of diluent into the tubes labeled 150, 75, 37.5, 18.75, 9.38, 4.69 and 2.34 ng/ml.
4. Pipette 250 µl of the 300 ng/ml myoglobin standard into the tube labeled 150 ng/ml and mix. This provides the 150 ng/ml myoglobin standard.
5. Similarly prepare the remaining standards by serial dilution.

[1] The SPARCL™ technology was developed by Lumigen Corp.

[2] The white SPARCL™ plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

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SAMPLE PREPARATION

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 frozen 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 serum from healthy Sprague Dawley rats contained myoglobin levels of 122±52 ng/ml (mean±SD, n =10, range = 11 – 154 ng/ml)

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 and 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.

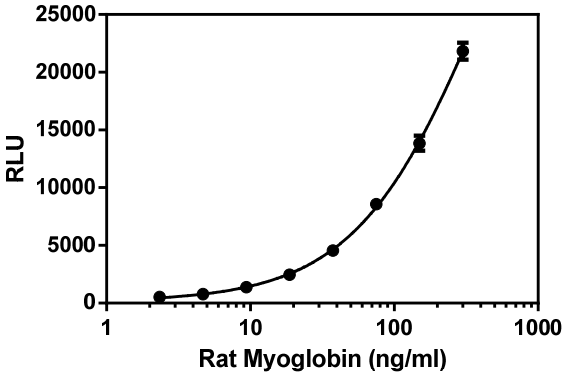
CALCULATION OF RESULTS

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. 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 $\log_{10}$ of myoglobin concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of myoglobin 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 myoglobin 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 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.

Myoglobin (ng/ml)	RLU
300	21822
150	13853
75	8575
37.5	4546
18.75	2461
9.38	1379
4.69	788
2.34	529



REFERENCES

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

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