HUMAN CARDIAC H-FABP SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: HFABP-SP-20

FOR RESEARCH USE ONLY

INTRODUCTION

Fatty acid-binding proteins are ~15 kDa cytoplasmic proteins involved in fatty acid transport and metabolism. The isoform expressed in heart (H-FABP) is highly abundant, representing 10-20 mol % of total cytoplasmic protein. H-FABP is released into blood after cardiac injury. H-FABP is expressed in both cardiac and skeletal muscle. When used as a cardiac biomarker, it is therefore important to rule out skeletal muscle injury. In the absence of cardiac injury, H-FABP can also be used as a biomarker of skeletal muscle damage.

PRINCIPLE OF THE ASSAY

The human H-FABP SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two different H-FABP antibodies. One is conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When the HRP and acridan conjugates bind to H-FABP they are brought into 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 (not bound to H-FABP) produce no signal. This principle allows the development of a homogeneous assay that allows rapid measurement of H-FABP.

Standards and diluted samples are mixed with HRP and acridan-conjugates in the wells of the 96-well SPARCL™ plate[2] provided with the kit. 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 H-FABP is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-FABP HRP conjugate **Store ≤** -70°C
- Anti-FABP acridan conjugate **Store ≤** -70°C
- FABP 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:

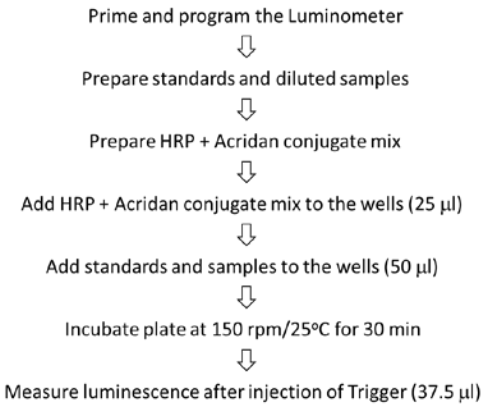
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP conjugate, acridan conjugate and FABP 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.

GENERAL INSTRUCTIONS

1. This kit is for research use only. It must not be used for diagnostic testing of human samples.
2. Please take the time to completely read all instructions before starting your assay. Contact us if you need clarification.
3. All reagents used in the assay should be allowed to reach room temperature (25°C) before use.
4. 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 have a maximum volume of 300 µl.
5. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The FABP stock is comprised of pure H-FABP in a stabilizing carrier protein matrix.

1. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml.
2. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 50 ng/ml standard.
3. Dispense 150 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml.
4. Pipette 150 µl of the 50 ng/ml standard into the tube labeled 12.5 ng/ml and mix. This provides the 25 ng/ml standard.
5. Similarly prepare the remaining standards by two-fold serial dilution.

[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.

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SAMPLE PREPARATION

We found H-FABP levels ranging from <5 ng/ml to >300 ng/ml. Because of the wide range of possible values, we cannot recommend a single dilution that is appropriate for all samples. To avoid matrix effects, samples must be diluted at least 16-fold. Only use the diluent provided with the kit for dilution of samples. Ascites fluid, serum, lithium heparin plasma and K3-EDTA plasma can be tested with this assay.

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 thawing, briefly centrifuge to ensure that the contents are at the bottom of the tubes. 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) 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 triplicate).
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.

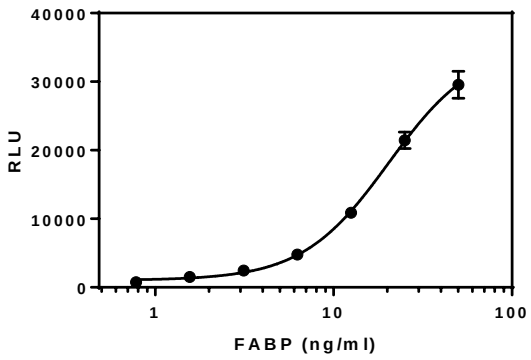
CALCULATION OF RESULTS

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

H-FABP (ng/ml)	RLU
50	29539
25	21444
12.5	10892
6.25	4769
3.13	2466
1.56	1524
0.78	739



REFERENCES

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

Rev 030619

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