# MONKEY CRP SPARCL™ ASSAY
# Life Diagnostics, Inc., Catalog Number: CRP-SP-3

## INTRODUCTION

CRP (C-reactive protein) is an acute phase protein that is elevated in monkey serum because of injury, infection, and disease. Normal levels of CRP range from 0-8.3 μg/ml (refs 1-2) and can increase >100 fold during the acute phase response (refs 1-8).

## PRINCIPLE OF THE ASSAY

The monkey CRP SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 9) assay uses two different affinity purified CRP-specific antibodies that are used in our preclinical CRP ELISA kits (refs 5-8). One is conjugated to horse radish peroxidase (HRP) and the other is conjugated to acridan, a chemiluminescent substrate. When HRP and acridan conjugated CRP antibodies bind to CRP 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 CRP 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[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 CRP is proportional to luminescence and is derived from a standard curve.

## MATERIALS AND COMPONENTS

**Materials provided with the kit:**

- Anti-CRP HRP conjugate **Store ≤ -70°C**
- Anti-CRP acridan conjugate **Store ≤ -70°C**
- Monkey CRP stock (2 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:**

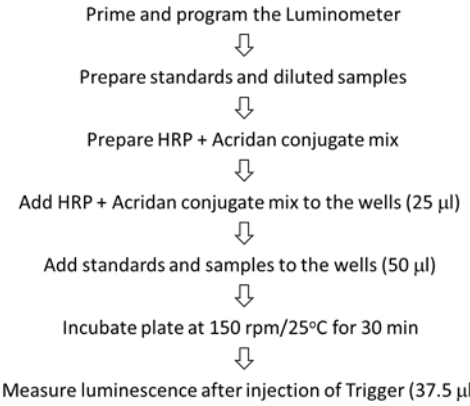
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Graphing software

## STORAGE

Store the HRP conjugate, acridan conjugate and CRP stock at or below -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 4°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 monkey CRP stock is comprised of lyophilized CRP diluted in a protein matrix. The CRP content was determined by reference to purified monkey CRP prepared at Life Diagnostics, Inc.

1. Reconstitute the lyophilized stock with diluent as described on the vial label. Mix gently until dissolved.
2. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the CRP reference standard vial label. Then add the indicated volume of CRP standard and mix gently. This provides the 250 ng/ml standard.
4. Dispense 150 μl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
5. Pipette 150 μl of the 250 ng/ml CRP standard into the tube labeled 125 ng/ml and mix. This provides the 125 ng/ml CRP standard.
6. Similarly prepare the remaining standards by serial dilution.

Use the standards within 30 minutes 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.

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## SAMPLE PREPARATION

Serum or plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same). If serum samples cannot be assayed immediately, they should be frozen at –70°C. Avoid repeated freeze-thaws.

We found that CRP is present in normal monkey serum at a concentration of approximately 5 μg/ml. Acute phase concentrations can exceed 150 μg/ml. We suggest that samples initially be diluted 1000-fold using the following procedure for each sample to be tested:

1. Dispense 195 μl and 192 μl of diluent into separate tubes.
2. Pipette 5.0 μl of serum or plasma into the tube containing 195 μl of diluent using a precision micro pipettor and mix. This provides a 40-fold diluted sample.
3. Pipette 8.0 μl of the 40-fold diluted sample into the second tube containing 192 μl of diluent and mix. This provides a 1000-fold dilution of the original sample.

To avoid matrix effects, samples must not be tested at dilutions less than 125-fold. Use the samples within 30 minutes.

## 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 several 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 4°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.

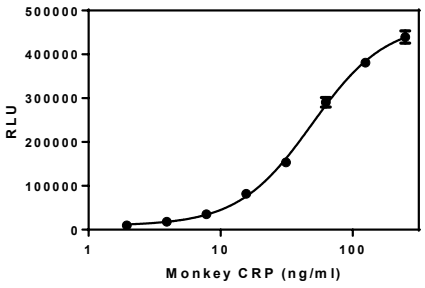
## CALCULATION OF RESULTS

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

| CRP (ng/ml) | RLU |
|---|---|
| 250 | 439524 |
| 125 | 380864 |
| 62.5 | 290618 |
| 31.25 | 153572 |
| 15.63 | 81738 |
| 7.81 | 35273 |
| 3.91 | 18035 |
| 1.95 | 9707 |



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