

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 as a result 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 kit². 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-monkey CRP HRP conjugate stock. **Store ≤ -70°C**
- Anti-monkey CRP acridan conjugate stock. **Store ≤ -70°C**
- Monkey CRP stock (2 vials). **Store ≤ -70°C**
- Diluent (CSD50-1), 50 ml
- Trigger solution, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Micro-Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC 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 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

All reagents used in the assay should be allowed to reach room temperature (25°C) before use.

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 tubes³ 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.

Please Note: Use the standards within 30 minutes of preparation.

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 ~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.

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

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.

³ Although tubes can be used to prepare standards, we recommend that dilutions be performed in wells A1-A8 of the clear untreated 96-well plate provided with the kit. This allows rapid transfer of standards to the white SPARCL plate using a multipipettor.

Diluted samples can also be first aliquoted into appropriate wells of the clear polystyrene plate and subsequently transferred to the SPARCL™ plate with a multipipettor. If using this method, ensure that an excess volume is aliquoted into the clear plate in order to ensure complete transfer of 50 µl aliquots to the SPARCL™ plate

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 μ l 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 μ l of conjugate mix into each well.
4. Dispense 50.0 μ l 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 μ l).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

CALCULATION OF RESULTS

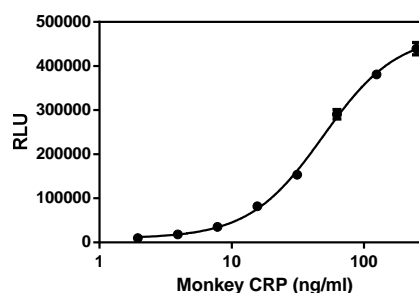
1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the log₁₀ of the CRP concentration.
3. Fit the data to using a sigmoidal four-parameter logistic equation.
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 actual concentration of CRP in the serum or plasma 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 CRP 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 in 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



REFERENCES

1. Jinbo T, Hayashi S, Iguchi K, Shimizu M, Matsumoto T, Naiki M, and Yamamoto S. Development of monkey C-Reactive protein method. *Vet Immunol Immunopathol.* 61:195-202 (1998)
2. Jinbo T, Ami Y, Suzuki Y, Kobune F, Ro S, Naiki M, Iguchi K, Yamamoto S. Concentrations of C-reactive protein in normal monkeys (*Macaca irus*) and in monkeys inoculated with *Bordetella bronchiseptica* R-5 and measles virus. *Vet Res Commun.* 23:265-74 (1999)
3. Hart BAT, Bank RA, De Roos JADM, Brok H, Jonker M, Theuns HM, Kakami J and Te Koppele JM. Collagen-induced arthritis in rhesus monkeys: evaluation of markers for inflammation and joint degradation. *British J. Rheumatology* 37:314-323 (1998)
4. Kingstrom J, Plyusnin A, Vaheri A and Lundkvist A. Wild-type Puumala Hanataivirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in *Cynomolgus* Macaques. *J. Virology.* 76:444-449 (2002)
5. Ossetrova NI, et. al. The use of discriminant analysis for evaluation of early-response multiple biomarkers of radiation exposure using non-human primate 6Gy whole body radiation model. *Radiation Measurements* 42:1158-1163 (2007)
6. Blackwood RS, et. al. Effects of the Macrolide drug tylosin on chronic diarrhea in Rhesus Macaques (*Macaca mulatta*). *Comparative Medicine* 58(1):81-87 (2008)
7. Blakely WF, et. al. Multiple parameter radiation injury assessment using a nonhuman primate radiation model-biodosimetry applications. *Health Phys.* 98(2):153-159 (2010)
8. Ossetrova NI, Sandgren DJ and Blakely WF. C-reactive protein and serum amyloid A as early-phase and prognostic indicators of acute radiation exposure in nonhuman primate total-body irradiation model. *Radiation Measurements* 46:1019-1024 (2011)
9. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. *J Am Chem Soc.* 20;135(11):4191-4 (2013)

Rev 072716

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