HUMAN CRP SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: CRP-SP-20

FOR RESEARCH USE ONLY

INTRODUCTION

CRP (C-reactive protein) is an acute phase protein that is synthesized in the liver and secreted into blood. It is a pentamer, comprised of identical 25 kDa subunits. During the acute phase response, as a result of infection, disease or tissue trauma, serum levels can increase >100-fold.

PRINCIPLE OF THE ASSAY

The human CRP SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay allows measurement of CRP using a single 30-minute incubation that requires no wash steps. The assay uses two different CRP-specific antibodies. One is conjugated to horse radish peroxidase (HRP); 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-human CRP HRP conjugate stock. **Store ≤ -70°C**
- Anti-human CRP acridan conjugate stock. **Store ≤ -70°C**
- Human CRP stock. **Store ≤ -70°C**
- Diluent (CSD50-1), 2 x 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 at least two weeks). 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 human CRP stock is comprised of highly purified human CRP diluted in a carrier protein matrix.

1. Thaw the CRP stock shortly before use.
2. Label 8 polypropylene tubes[3] as 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the CRP stock vial label. Then add the indicated volume of CRP stock and mix gently. This provides the 50 ng/ml standard.
4. Dispense 150 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
5. Pipette 150 µl of the 50 ng/ml CRP standard into the tube labeled 25 ng/ml and mix. This provides the 25 ng/ml CRP standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Please Note: Use the standards within 30 minutes of preparation. Store unused CRP stock at or below -70°C if future use is intended.

SAMPLE PREPARATION

Because the human CRP SPARCL™ assay uses a homogenous format, a prozone or hook effect can occur at high CRP concentrations. Because serum CRP levels can range from 2 – 100 µg/ml, depending on the degree of the acute phase response, it is not possible to recommend a single dilution that can be used for all samples. We **suggest** that samples initially be tested at dilutions of 500-, 5,000- and 50,000-fold:

1. Dispense 998 µl of diluent into one tube and 180 µl of diluent into two tubes.
2. Pipette 2.0 µl of serum or plasma into the tube containing 998 µl of diluent and mix. This provides a 500-fold dilution.
3. Pipette 20.0 µl of the 500 fold diluted sample into the second tube containing 180 µl of diluent and mix. This provides a 5,000-fold dilution.
4. Pipette 20.0 µl of the 5,000-fold diluted sample into the third tube containing 180 µl of diluent and mix. This provides a 50,000-fold 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.

[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

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

Use the samples within 30 minutes.
Optimal dilutions of culture supernatants and tissue extracts must be determined empirically.

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 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 (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

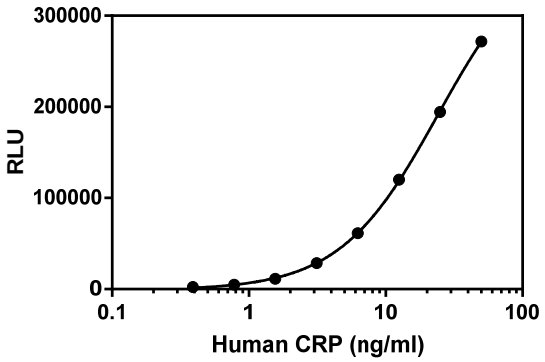
CALCULATION OF RESULTS

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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the $\log_{10}$ 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.
7. If a prozone effect is evident, for example when RLU values for samples tested at a 500-fold dilution are lower than those obtained at a 5,000-fold dilution, eliminate such values from analysis.

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
50	271529
25	194480
12.5	120046
6.25	61422
3.13	28649
1.56	11282
0.78	4829
0.39	2341



REFERENCES

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

Rev 101016
For technical assistance please email us at
techsupport@lifediagnostics.com