TECHNICAL MANUAL

Human CRP NovoLISA

(de Novo protein Linked Instantaneous Solution-based Assay)

For the quantitative determination of human C-Reactive protein (CRP) concentrations in cell culture supernatants and serum.

INSTRUCTIONS FOR USE

NL0101

This package insert must be read in its entirety before using the product.

For research use only. Not intended for diagnostic use.

Rev. 1.001

1. Kit Summary

Target:	Pentameric CRP
Detection method:	Luminescent
Sample types:	Serum and cell culture supernatants
Reactive species:	Human
Dynamic range:	0.02–20 mg/L
Reportable range:	1.3–1,000 mg/L (for serum samples)
Assay time:	15 min
Limit of detection:	0.515 mg/L (for serum samples)
Sample volume:	5 µL

2. Components & Storage Conditions

The table below lists provided item components with their part numbers, units provided, and volumes:

Component Name	Part Number	Units	Volume
10x Sensor	NL0101A	1	500 µL
LuxSit™ Pro Substrate	NL0101B	1	100 µL
10x Assay Buffer	NL0101C	1	5 mL
Human CRP Standard (1000 mg/L)	NL0101D	1	20 µL
96-well white opaque plate	-	1	-

Materials required, but not provided:

- Multichannel pipette(s)
- Distilled, deionized or Milli-Q water
- Dilution tubes, reagent reservoir trays, etc.
- Microtiter plate reader capable of reading luminescence

Components should be stored as follows upon receipt:

Store complete kit at -80°C upon receipt. Alternatively, store induvial components as follows:

- Store **CRP Sensor** at -80°C.
- Store **LuxSit™ Pro Substrate** and **Human CRP Standard** at -20°C. ***Limit light exposure****
- Store **10x Assay Buffer** at room temperature.

Product is stable for at least 6 months stored using either storage scenario.

3. Overview

Monod Bio has developed a homogenous *de novo* protein assay system based on Split LuxSit™ Luciferase technology for the quantitative detection of human C-Reactive protein. De novo protein design enables the creation of proteins with customized functions and enhanced stability, providing innovative tools that accelerate research through more efficient and precise assays. CRP, a highly conserved member of the pentraxin protein family, plays a crucial role in the innate immune system [1]. It is secreted by the liver in response to inflammatory cytokines, with levels rising rapidly during conditions such as tissue injury, infection, and inflammation. As a result, CRP measurements have been widely used in clinical settings [2]. CRP is involved in the recognition and clearance of pathogens, activation of the classical complement pathway, and stimulation of phagocytic cells for the removal of cellular debris [3].

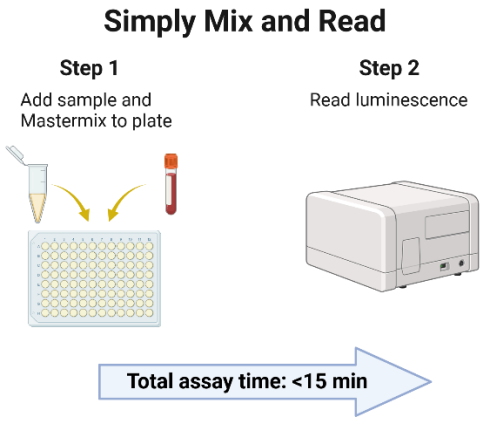


Figure 1. Assay workflow. The LuxSit™ Human C-Reactive Protein/CRP Immunoassay is a 15-minute no-wash mix and read assay designed to quantify CRP in serum samples and cell culture supernatants.

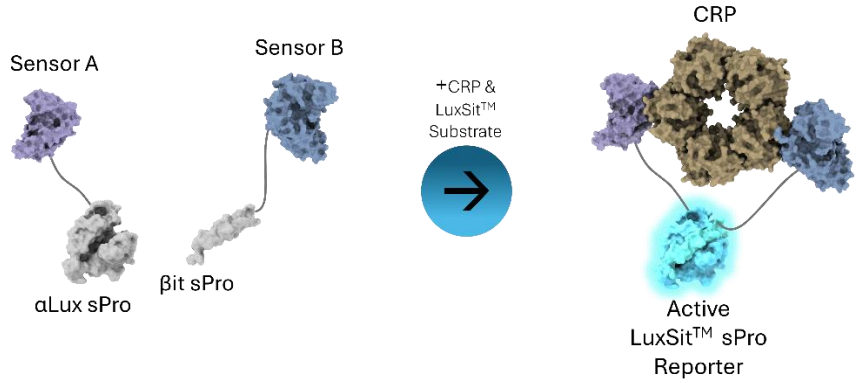
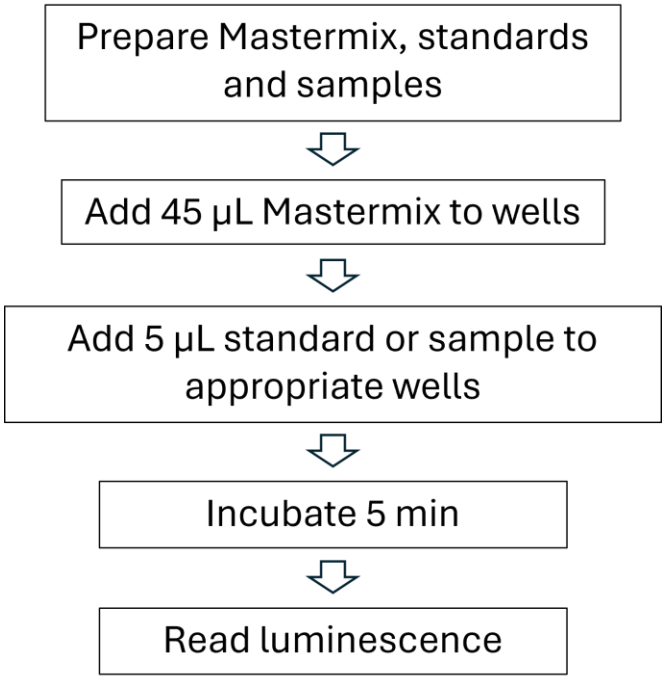


Figure 2. Assay principle. The assay utilizes two CRP-specific protein binders engineered with Monod's advanced AI/ML platform to precisely target CRP, minimizing cross reactivity. Each binder is linked to components of the LuxSit™ platform. Upon sample addition, the CRP-specific binders bind CRP, bringing the LuxSit™ components into close proximity and forming an active enzyme. This enzyme then reacts with the substrate to produce luminescence.

4. Assay at a glance



5. Before you begin

- Thaw CRP Biosensor A and CRP Biosensor B at room temperature in preparation for assay and mix each thoroughly prior to use by pipetting 3 to 5 times.

- Allow LuxSit™ Substrate to equilibration to room temperature prior to use in assay.

- Collect and prepare samples. Below are suggested collection conditions for common sample types.

    - **Serum samples:** Use serum separator tubes and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at *1000 x g*. Remove serum and assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

    - **Cell Culture Supernatants:** We recommend using 1X DMEM supplemented with 5% fetal bovine serum (FBS). The use of media with phenol red has minimal interference on the luminescent signal but does not significantly impact assay sensitivity. Remove particulates by centrifugation (300 *x g* for 5 min) and assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

6. Split LuxSit™ CRP Biosensor – 96 well Plate Protocol

The following protocol is detailed for a 96-well plate. Adjust volumes accordingly based on experimental requirements.

- **Pre-dilute samples**

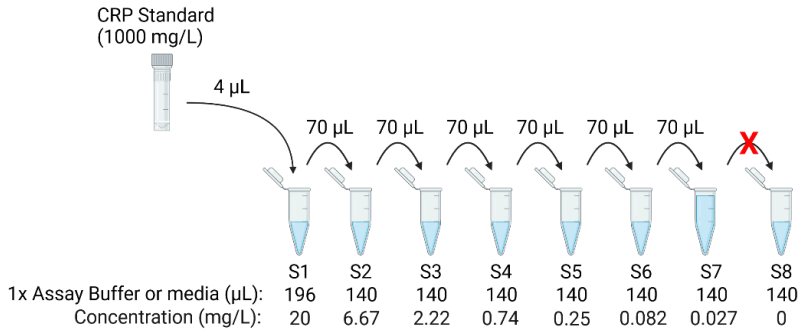
  See Sample Collection section (section 5, page 4) for suggestions on collecting samples.

  - If using **serum** samples:
    - Dilute **10x Assay Buffer** to **1x Assay Buffer** using deionized, distilled or Milli-Q water. For example, to make 10 mL add 1 mL of **10x Assay Buffer** to 9 mL of water.
    - Dilute each sample 50-fold into **1x Assay Buffer** to minimize matrix effects. For example, add 4 µL **sample** to 196 µL **1x Assay Buffer**.
  - **Cell culture supernatants** do not need to be diluted to be compatible with the assay. However, sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application. Testing samples neat and diluted 10, 100, and 1,000-fold may be an effective strategy. Use clean cell culture media to dilute cell culture supernatant samples.

- **Dilute CRP standards**

  *For serum samples prepare the standard curve dilutions using **1x Assay Buffer**. For cell culture supernatants prepare the standard curve dilutions using clean cell culture media. Aliquot unused **Human CRP Standard** and refreeze at -20°C. Do not refreeze these aliquots. Do not reuse standard dilutions.*

  - In a tube or microwell labeled S1, add 4 µL of **Human CRP Standard** to 196 µL of **1x Assay Buffer** (for serum samples) or **cell culture media** (for cell culture supernatant samples). Mix thoroughly.
  - Label additional tubes or microwells as S2, S3, S4, S5, S6, S7, and S8.
  - Add 140 µL of **1x Assay Buffer** (for serum samples) or **cell culture media** (for cell culture supernatant samples) to each labeled tube or microwell.
  - Perform 3-fold serial dilutions from the standard solution in S1 into the remaining tubes or microwells as follows:
    - Transfer 70 µL from S1 to S2. Mix thoroughly.
    - Repeat this process through S7, making sure to mix thoroughly after each transfer.



- Prepare Mastermix
  - Calculate the total number of wells to be used (standard curve and test samples) and prepare **Mastermix**. The following table provides recipes for commonly used volumes. *Adjust volumes based on experimental requirements.* ***LuxSit substrate*** *can be refrozen and thawed up to ten times. Do not refreeze* ***10x Sensor****. Store thawed* ***10x Sensor*** *at 4°C for up to four weeks.*

Component	96 wells	48 wells	24 wells
10x Assay Buffer	500 µL	250 µL	125 µL
10x Sensor	500 µL	250 µL	125 µL
LuxSit™ Pro Substrate	100 µL	50 µL	25 µL
Water	3,900 µL	1,950 µL	975 µL
Total Volume	**5 mL**	**2.5 mL**	**1.25 mL**

- Add Mastermix, Standards and Samples to plate
  - Transfer 45 µL of **Mastermix** to appropriate wells in the assay plate.
  - Add 5 µL of each of **standard** or **prediluted sample** to the appropriate wells (see example plate map below). It is recommended to run each standard and prediluted sample in duplicate.

	CRP Standards		Test Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1										
B	S2	S2										
C	S3	S3										
D	S4	S4										
E	S5	S5										
F	S6	S6										
G	S7	S7										
H	S8	S8										

- Read plate
  - Use either of the following protocols to read luminescent signals.

    End Point Protocol
    - Transfer samples to luminescence plate reader.
    - Mix sample for 3–5 second using orbital shaking.
    - Incubate 5 min. *Do not wait more than 20 minutes before reading samples.*
    - Read luminescence.

    Kinetic Protocol
    - Transfer samples to luminescence plate reader.
    - Mix sample for 3–5 seconds using orbital shaking.
    - Read luminescence for every well 1 minute for at least 15 minutes.

7. Interpretation of Results

- If using *Kinetic Read Protocol* plot luminescence over time and identify the plateau or maximal luminescent reading to use for subsequent analysis (see Figure 3A).
- Subtract background luminescence from all standards and samples by subtracting the average luminescence in the S8 (0 ng/mL CRP) wells.
- Create a standard curve by plotting luminescence versus CRP concentration. Fit the data with nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope).
- Use the standard curve to interpolate the CRP concentration of test samples. If samples have been diluted, the **concentration should be multiplied by the dilution factor**. For example, **serum** samples should be adjusted by **50x** to account for dilutions made on page 6.

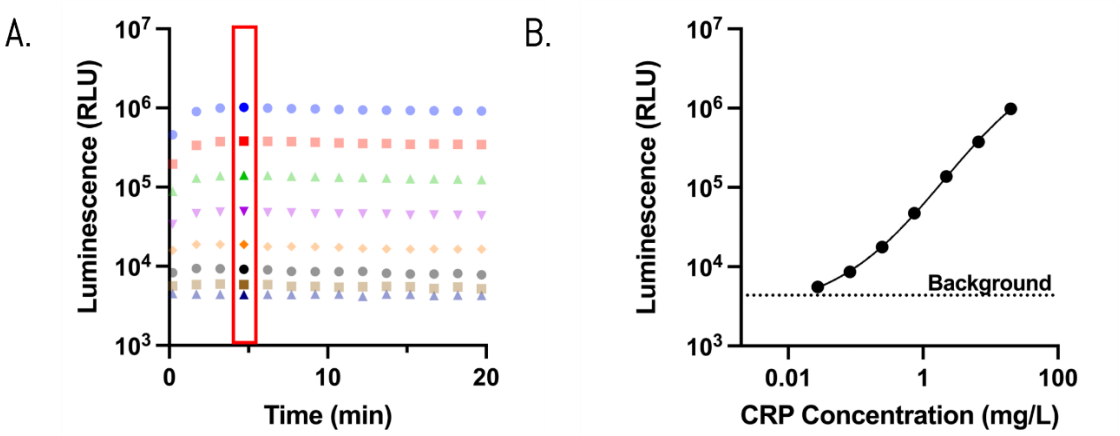


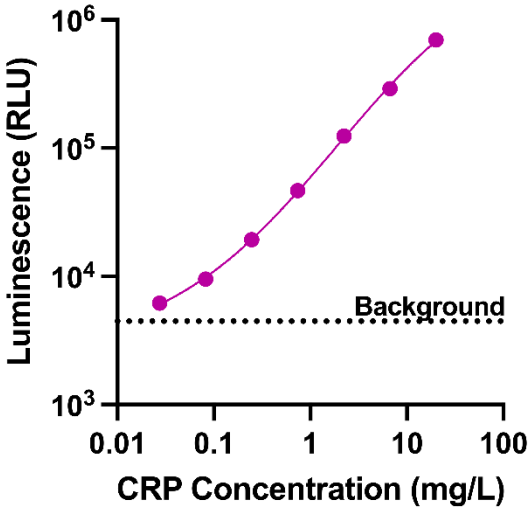
Figure 3. (A.) Example data using the *Kinetic Read Protocol*. Plateau values to be used for further analysis are indicated in the red box. **(B.)** Example standard curve plot using the plateau values from A.

8. Plate Reader/Luminescent Considerations

It is important to consider that there may be significant differences between plate reader/luminometers (even from the same manufacturer) resulting in different relative luminescent unit (RLU) output values from equivalent experimental assay workflows. RLU output values can be modified by changing instrument integration time, gain, and read height within the settings of each plate reader/luminometer software. Users should consider the impact of each of these settings when determining the ideal instrument configuration for their experiment.

9. Performance Characteristics

Interpolated values for **Performance Characteristics** were generated from a 4PL curve fit of the **Human CRP Standard** serial diluted in **1x Assay Buffer** (serum samples) or DMEM + 5% FBS (mock cell culture supernatant) and corrected for any dilutions. Below is an example of a 4PL curve fit for the CRP Standard diluted in DMEM + 5% FBS.



Precision

Intra Assay Precision (within an assay)

Three unique patient serum samples of known concentrations were tested using eight technical replicates of each to assess intra-assay precision.

Inter-Assay Precision (between assays)

Three unique patient serum samples of known concentrations were tested using eight technical replicates of each per plate across three separate assays to assess inter-assay precision.

The following table reports precision results:

Precision Type	Mean Interpolated Value	%CV
Intra-Assay (n = 8), Serum Sample 1	432 mg/L	3.0%
Intra-Assay (n = 8), Serum Sample 2	63 mg/L	2.9%
Intra-Assay (n = 8), Serum Sample 3	14 mg/L	4.3%
Inter-Assay (n = 3 plates), Serum Sample 1	413 mg/L	9.7%
Inter-Assay (n = 3 plates), Serum Sample 2	62 mg/L	8.9%
Inter-Assay (n = 3 plates), Serum Sample 3	13 mg/L	16.5%

Recovery:

Three different concentrations of CRP Standard were spiked into cell culture supernatant or a diluted serum sample within the dynamic range of the assay. The following table reports recovery results:

Sample Type	Average Recovery	Range of Recoveries
Cell Culture Supernatant (DMEM with 5% FBS)	96%	92–101%
50-Fold Diluted Serum Sample	103%	102–105%

Linearity

The linearity of serum samples was assessed by serially diluting a sample using Assay Buffer within the dynamic range of the assay. The following table reports linearity results:

Dilution Factor	Interpolated Value	Patient Serum Sample
Original Dilution	mg/L	375.28
2-Fold Further	mg/L	192.82
	Percent of Expected	**103%**
4-Fold Further	mg/L	104.36
	Percent of Expected	**111%**
8-Fold Further	mg/L	54.23
	Percent of Expected	**116%**
16-Fold Further	mg/L	23.80
	Percent of Expected	**101%**

The linearity of cell culture media was assessed by spiking CRP standard into and serial diluting in DMEM with 5% FBS within the dynamic range of the assay. The following table reports linearity results:

Dilution Factor	Interpolated Value	DMEM with 5% FBS
Original Spike	mg/L	262
3-fold	mg/L	90
	Percent of Expected	**103%**
9-fold	mg/L	31
	Percent of Expected	**107%**
27-fold	mg/L	11
	Percent of Expected	**115%**
81-fold	mg/L	3.6
	Percent of Expected	**111%**

10. References

1. Du Clos, T. W. (2000). Function of C-reactive protein. *Annals of medicine*, *32*(4), 274-278.
2. Black, S., Kushner, I., & Samols, D. (2004). C-reactive protein. *Journal of Biological Chemistry*, *279*(47), 48487-48490.
3. Nehring, S. M., Goyal, A., & Patel, B. C. (2017). C reactive protein.