

# N-geneous™ Wide Range CRP Reagent

## INTENDED USE

For the quantitative measurement of C-Reactive Protein (CRP) concentration in serum or plasma.

## SUMMARY

CRP is an acute phase protein that is elevated during inflammation and tissue necrosis. Measurement of CRP is useful for determining the existence of inflammatory lesions<sup>1</sup> and to monitor treatment.

## PRINCIPLE

The N-geneous™ Wide Range CRP Reagent is an enhanced latex-agglutination turbidimetric immunoassay. Sample is added to a buffer solution and mixed with a suspension of mouse anti-human CRP monoclonal antibody that is bound to latex. CRP binds to the latex-bound antibody and agglutinates. The light scattering caused by the increase in particle size is used as a measure of CRP concentration. The amount of light scattering is proportional to the concentration of CRP in the sample.

## REAGENTS

### Composition

Component	Ingredients	Concentration
Reagent 1	Buffer Preservative	pH 8.5
Reagent 2	Mouse anti-human CRP monoclonal antibody-coated latex	2 mg/mL

### Precautions and Warnings

1. For In Vitro Diagnostic Use.
2. Do not use the reagents beyond the expiration date printed on the label.
3. **Warning:** All specimens used in the test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.<sup>2</sup>
4. N-geneous™ Wide Range CRP Reagents must be used with N-geneous™ Wide Range CRP Calibrator.
5. **Caution:** Avoid freezing reagents

### Preparation

Reagent 1: Liquid, ready to use.

Reagent 2: Liquid, ready to use. Mix by inversion prior to placing on analyzer.

### Storage and Stability

Unopened reagent is stable until the expiration date shown on the label when stored at 2 - 8°C.

Once opened, the reagent is stable up to 90 days at 2 - 8°C or 7 days at 20 - 25°C.

DO NOT FREEZE.

### Indications of Deterioration

Presence of turbidity or microbial growth may indicate deterioration.

Inability to recover control values.

## SPECIMEN COLLECTION AND PREPARATION

Serum, EDTA-plasma, and sodium or lithium heparinized-plasma are the recommended collection media. Use standard sample collection and preparation methods.<sup>3</sup>

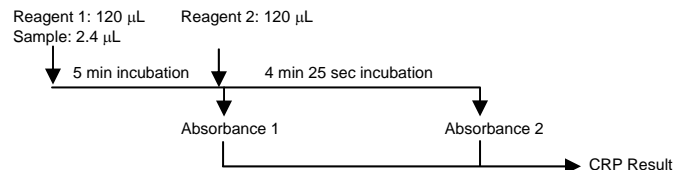
If not analyzed promptly, serum or plasma specimens may be stored at 2-8°C for 14 days, or at 20 - 25°C for 5 days. If specimens need to be stored for more than 14 days, they may be preserved at -20°C or below for 1 month.

Samples may be frozen and thawed twice.

## PROCEDURE

### Assay

Below is a general example of the N-geneous™ Wide Range CRP assay procedure for an automated analyzer. All analyzer applications should be validated.



For assistance with applications on automated analyzers, please contact Genzyme Diagnostics Technical Marketing at (800) 332-1042.

### Materials Provided

N-geneous™ Wide Range CRP Reagents 1 and 2 are required for the measurement of CRP. The N-geneous™ Wide Range CRP reagents are packaged and sold separately. Either of the following items may be included in the package you receive.

Description	Configuration	Catalog Number
N-geneous™ Wide Range CRP Reagent 1	25 mL	80-6640-00
N-geneous™ Wide Range CRP Reagent 2	25 mL	80-6641-00

## Materials Required but not Provided:

Description	Configuration	Catalog Number
N-geneous™ Wide Range CRP Calibrator Set	5 Levels x 2 mL vials	80-6655-00

- Quality Control materials.
- Analyzer capable of running two reagent chemistries.

### Calibration

Only the N-geneous™ Wide Range CRP Calibrators should be used to calibrate the N-geneous™ Wide Range CRP assay. The assigned values of the N-geneous™ Wide Range CRP Calibrators are traceable to CRM470 BCR reference material.<sup>4</sup>

Refer to the instrument operator's manual for analyzer specific calibration procedures and for guidance in determining calibration frequency.

### Quality Control

Reliability of test results should be monitored routinely with quality control materials or serum pools that reasonably represent performance with patient specimens. Controls or serum pools should be used to monitor that the reagents are functioning properly and that correct procedures are being followed. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range, follow normal troubleshooting procedures. If assistance is required, please call Genzyme Technical Marketing (800) 332-1042.

Quality control requirements should be established in accordance with local, state and/or federal regulations, or accreditation requirements.

## RESULTS

To convert from mg/L to mg/dL, divide the mg/L value by 10.

### Limitations/Interfering Substances

All interference studies were conducted according to NCCLS guideline EP7.<sup>5</sup>

Intralipid® concentrations greater than 0.8% (2400 mg/dL triglyceride equivalent) showed a bias of greater than 0.3 mg/L at approximately 3 mg/L CRP.

Refer to the work of Young<sup>6</sup> for a review of the effects of drugs on clinical laboratory tests.

Heterophilic antibodies: Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.<sup>7</sup>

Due to the large range of specimen concentrations possible, sample probe washing must be adequate to prevent sample carryover.

### Expected Values

Each laboratory should confirm the reference interval for the patient population it serves.

Samples from 50 apparently healthy individuals were tested with the N-geneous™ Wide Range CRP assay.<sup>8</sup> The results were consistent with the literature values for the 5<sup>th</sup> and 95<sup>th</sup> percentiles of 0.19 and 9.14 mg/L for females and 0.28 and 8.55 mg/L for males.<sup>9</sup>

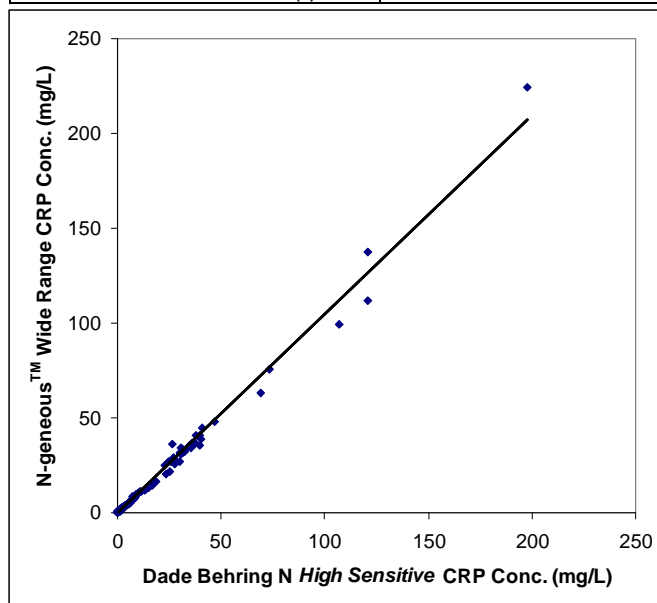
## SPECIFIC PERFORMANCE CHARACTERISTICS

### Accuracy

Comparative performance studies were conducted using the N-geneous™ Wide Range CRP Reagent on the Roche Hitachi 912 clinical analyzer and the Dade Behring N *High Sensitive* CRP method. 229 serum samples, with CRP concentrations between 0.2 and 198 mg/L were tested over 6 days. The protocol followed the recommendations of NCCLS EP9.<sup>10</sup>

The regression analysis is provided below:

N-geneous™ Wide Range CRP vs. Dade Behring N <i>High Sensitive</i> CRP (n = 229)	
Slope	1.05
Intercept (mg/L)	-0.31
Correlation Coefficient (r)	0.995



## Precision

Precision of the N-geneous™ Wide Range CRP Reagent was determined using 5 levels of frozen pooled human serum following NCCLS EP5<sup>11</sup> by running samples in duplicate, twice per day for 20 days on the Roche Hitachi 912 analyzer.

### Within-Run Precision

Serum pool / Control	Mean Recovery (mg/L)	Standard Deviation (mg/L)	CV
Level 1	0.30	0.02	5.5%
Level 2	1.00	0.02	1.8%
Level 3	2.97	0.04	1.3%
Level 4	51.3	0.61	1.2%
Level 5	202	3.0	1.5%

### Total Precision

Serum pool / Control	Mean Recovery (mg/L)	Standard Deviation (mg/L)	CV
Level 1	0.30	0.02	6.7%
Level 2	1.00	0.02	2.3%
Level 3	2.97	0.05	1.7%
Level 4	51.3	0.96	1.9%
Level 5	202	3.1	1.5%

### Limit of Detection

The limit of detection is the concentration that is not statistically distinguishable from zero. Saline was run twenty times with N-geneous™ Wide Range CRP Reagent on the Roche Hitachi 912 clinical analyzer and the mean plus two standard deviations was used to define the detection limit: 0.04 mg/L.

### Specificity

The following substances at the concentrations shown did not affect performance of the CRP assay in a serum pool with approximately 3 mg/L CRP:

<u>Substance</u>	<u>Concentration Tested</u>
Bilirubin	60 mg/dL
Hemoglobin	1000 mg/dL
Ascorbic acid	500 mg/dL
Intralipid®	0.8% (2400 mg/dL trig)
Acetaminophen (paracetamol)	20 mg/dL
Acetylsalicylic acid	50 mg/dL
Ampicillin	5 mg/dL
Caffeine	10 mg/dL
Captopril	6 mg/dL
Chlorpheniramine maleate	0.8 mg/dL
Cimetidine	10 mg/dL
Cyclosporin U	0.8 mg/dL
Doxycycline hyclate	6 mg/dL
Furosemide	2 mg/dL
Ibuprofen	40 mg/dL

Indomethacin	1 mg/dL
Levodopa	160 mg/dL
Lovastatin	1.6 mg/dL
Methotrexate	450 mg/dL
Methyldopa	2.5 mg/dL
Metoprolol tartrate	0.3 mg/dL
Metronidazole	1 mg/dL
Nicotinic acid	2 mg/dL
Omeprazole	7.2 mg/dL
Prednisone	1.2 mg/dL
Promethazine hydrochloride	1 mg/dL
Propranolol hydrochloride	0.5 mg/dL
Quinidine sulphate	5 mg/dL
Simvastatin	0.8 mg/dL
Theophylline	25 mg/dL
Tolbutamide	100 mg/dL

A high dose hook effect was not observed at concentrations up to 1000 mg/L CRP.

Rheumatoid factor was tested to 1700 IU/mL and did not affect performance.

### Linearity

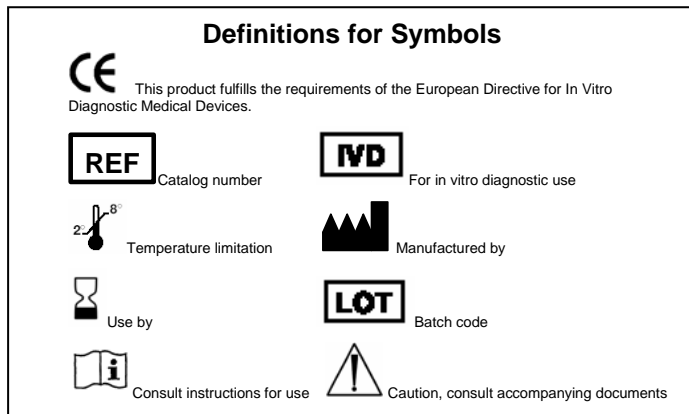
Using NCCLS protocol EP6,<sup>12</sup> the N-geneous™ Wide Range CRP method was linear from 0.04 to 320 mg/L. The samples were admixtures of low and high serum. The high serum was prepared by supplementing a pool with purified human CRP.

Specimens above 320 mg/L may be diluted with physiological saline. Multiply the result by the dilution factor to obtain the CRP concentration in the sample.

### References:

1. Burtis CA and Ashwood ER, Teitz Textbook of Clinical Chemistry, 2<sup>nd</sup> ed. Philadelphia: WB Saunders, 1994: 674, 713-714.
2. Richardson JH and Barkley WE, eds. Biosafety in Microbiological and Biomedical Laboratories, U.S. Dept. of Health and Human Services, Public Health Service, HHS Publication No. (CDC) 84-8395, Washington, DC: 1984.
3. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens: Approved Guideline. NCCLS document H18-A Villanova, PA: 1990.
4. Kimberly MM et al., Standardization of Immunoassays for Measurement of High-Sensitivity C-Reactive Protein. Phase I: Evaluation of Secondary Reference Materials. Clin Chem 2003; 49: 611-619.
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7. Boscato LM and Stuart MC, Heterophilic Antibodies: A Problem for All Immunoassays. Clin Chem 1988; 34: 27-33.
8. National Committee for Clinical Laboratory Standards. How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline. NCCLS document C28-A2, Villanova, PA:2002.
9. Rifai N and Ridker PM, Population Distributions of C-reactive Protein in Apparently Healthy Men and Women in the United States: Implication for Clinical Interpretation. Clin Chem 2003; 49: 666-669.
10. National Committee for Clinical Laboratory Standards. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline. NCCLS document EP9-A. Villanova, PA:2002.
11. National Committee for Clinical Laboratory Standards. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. NCCLS document EP5-A. Villanova, PA:1999.
12. National Committee for Clinical Laboratory Standards. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A. Villanova, PA:2003.



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February 2, 2004  
80-6622-00-00

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