

Extended Range C-Reactive Protein (erCRP) Reagent

Immunturbidimetric Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Specimen Type	:	Serum or plasma
Method	:	Sample Blank/Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of C-Reactive Protein (CRP) concentration in serum or plasma on automated clinical chemistry analyzers.

CLINICAL SIGNIFICANCE^{1,2,3,4}

C-Reactive protein is an acute phase protein which, in the presence of Calcium, is able to bind to the polysaccharides present in many bacteria, fungi, protozoal parasites and endogenous polyanions such as nucleic acids. CRP has a role in the non specific immune response by initiating the complement pathway and phagocytosis.

CRP also has a role in the removal of endogenous material resulting from cell damage. CRP is recognised as one of the most sensitive acute phase proteins, and therefore can be beneficial in the screening for organic disease, diagnosis and follow up of infectious disease, stroke, following surgery, detecting rejection in renal allograft recipients and managing neonatal septicaemia and meningitis. Furthermore, given the role that systemic inflammation may have in the pathogenesis of atherothrombosis, baseline CRP concentrations may also serve as a measure of the risk of future myocardial infarction or stroke.

METHODOLOGY

The Extended 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 to measure the CRP concentration. The amount of light scattering is proportional to the concentration of CRP in the samples.

REAGENT COMPOSITION

Active Ingredients

Reagent 1

Buffer pH 8.5
Preservative

Reagent 2

Mouse anti-human CRP monoclonal antibody-coated latex 2mg/mL

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the Thermo Extended Range CRP reagent Material Safety Data Sheet. **Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.**

REAGENT PREPARATION

Reagents are supplied ready to use. Mix by inversion prior to use.

STABILITY AND STORAGE

Prior to Use:

When stored at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Once Opened:

When stored at 2-8°C the reagent is stable for at least 90 days, or 7 days if stored at 25°C. DO NOT FREEZE REAGENTS.

Indications of Reagent Deterioration:

- Turbidity; and/or
- Failure to recover control values within assigned range.

SPECIMEN COLLECTION AND HANDLING⁵

Serum: Use non-haemolysed serum.

Plasma: Use EDTA, sodium or lithium heparin.

Storage: Serum or plasma specimens may be stored at 2-8°C for 14 days after collection. For longer storage samples should be frozen at -20°C. Frozen samples are stable for up to 1 month. Samples may be frozen and thawed twice.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION: CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		
REAG 1	Reagent 1 (R1)	REAG 2	Reagent 2 (R2)

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining a constant temperature (37°C), measuring an absorbance at 570 nm and with the capacity to measure and store a non linear standard curve.
- Analyser specific consumables, eg. sample cups.
- Normal and abnormal assayed control material.
- Thermo erCRP Calibrator Set (TR83201).

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	570 nm
Secondary Wavelength	800 nm
Assay Type	2 Point Endpoint
Direction	Increase
Sample : Reagent ratio	1:100
e.g. Sample vol	3 µL
Reagent 1 vol	150 µL
Reagent 2 vol	150 µL
Delay time (sample + R1)	300 seconds
Incubation Time	265 seconds
Reagent Blank Limits	Low 0.000 AU
(570 nm / 800 nm, 1cm lightpath)	High 0.015 AU
Linearity	0.12 -320 mg/L
Limit of Quantification	0.12 mg/L
(570 nm / 800 nm, 1cm lightpath)	

CALCULATIONS

Results are automatically calculated by the analyser.

Final absorbance = Abs (Test) – Abs (Blank)

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. When using CRP to assess cardiovascular and peripheral vascular disease, results should be compared to previous values.
3. Unit conversion: mg/dL x 10 = mg/L

CALIBRATION

For calibration, only the Thermo Extended Range CRP Calibration Set (Catalogue number: TR83201) should be used. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. No "hook" affect was observed with samples containing CRP concentrations up to a level of 1000 mg/L.
2. Studies to determine the level of interference were carried out. The following results were obtained at a level of 3 mg/L CRP:
Intralipid®: No interference from triglycerides up to 2.7 mmol/L (240 mg/dL).
Bilirubin: No interference from bilirubin up to a level of 1026 µmol/L (60 mg/dL).
Haemoglobin: No interference from haemoglobin up to a level of 1000 mg/dL.
Ascorbic Acid: No interference from ascorbic acid up to a level of 28.4 mmol/L (500 mg/dL).
Rheumatoid Factor: No interference from rheumatoid factor up to a level of 1711 IU/mL.
3. For a more comprehensive review of factors affecting C-Reactive protein assays refer to the publication by Young.⁶
4. For information on interference by common exogenous substances please contact the Technical Support Group.
5. Heterophilic antibodies: Patients routinely exposed to animals or animal serum products can be prone to interference from heterophilic antibodies and anomalous values be observed⁷.

EXPECTED VALUES

Female: 0.19 - 9.14 mg/L
 Male: 0.28 - 8.55 mg/L

Expected value range determined using the 5th and 95th percentiles⁸.

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population it serves⁹.

PERFORMANCE DATA

The following was obtained using the Thermo Extended Range C-Reactive Protein Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on the specific analyser used.

IMPRECISION

Imprecision was evaluated over a period of 20 days using five levels of frozen pooled human serum and following the NCCLS EP5-A procedure.¹⁰

Within Run	Mean (mg/L)	SD (mg/L)	CV%
Level 1	0.3	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	Mean (mg/L)	SD (mg/L)	CV%
Level 1	0.3	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

ACCURACY

Comparison studies were carried out using a similar commercially available High Sensitive CRP reagent as a reference. Serum samples were assayed in parallel and the results were compared by least squares regression. The the following statistics were obtained:-

Number of sample pairs	229
Slope	1.03
Intercept	-0.25 mg/L
Correlation coefficient	0.995

LINEARITY

When run as recommended the linearity of the assay is dependent on the non linear calibration. Refer to instrument specific applications, and calibrator package insert. 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.

LIMIT OF QUANTIFICATION

When run as recommended the Limit of Quantification (LOQ), determined on the Hitachi 911® is 0.12 mg/L. Values below this should not be reported. The LOQ is the concentration of CRP at which the CV is 20%.


LIMIT OF DETECTION

When run as recommended the Limit of Detection (LOD), determined on the Hitachi 911® is 0.08 mg/L. The LOD is the concentration of CRP that can be distinguished from a saline blank, with 95% confidence.

REFERENCES

1. Silverman LM, Christnson RH. Amino Acids and Proteins in Tietz Textbook of Clinical Chemistry, Second Edition WB Saunders Company.
2. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. N Eng J of Med 1997;336:973-9.
3. Dahler-Eriksen BS, Lauritzen T, Lassen JF, et al. Near Patient Test for C Reactive Protein in General Practice: Assessment of Clinical, Organisational, and Economic Outcomes. Clin Chem 1999;45:478-85.
4. Whicher JT. Acute Phase Proteins, Physiology and Clinical Use. Clin Biochem Reviews 1990;11:4-9
5. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens: Approved Guideline. NCCKS document H18-A Villanova PA:1990
6. Young DS. Effects of Drugs on Clinical Laboratory Tests, Third Edition. AACCPress, 1990.
7. Boscato LM and Stuart MC, Heterophilic Antibodies: A problem for all immunoassays. Clin Chem 1988;34:27-33.
8. 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
9. Wachtel M et al. Creation and Verification of Reference Intervals. Laboratory Medicine 1995;26:593-7.
10. National Committee for Clinical Laboratory Standards. Precision Performance of Clinical Laboratory Devices, Approved Guideline-NCCLS; 1999, NCCLS Publication EP5-A.

© 2008 Thermo Fisher Scientific Inc. All rights reserved. Intralipid® is a registered trademark of Fresenius Kabi Nutrition AB, Sweden. Hitachi® is a registered trademark of Roche Diagnostics, Indianapolis, IN 46250. All other trademarks are the property of Thermo Fisher Scientific Inc and its subsidiaries.

 Fisher Diagnostics
 a division of Fisher Scientific Company, LLC
 a subsidiary of Thermo Fisher Scientific Inc.
 Middletown, VA 22645-1905 USA
 Phone: 800-528-0494
 540-869-3200
 Fax: 540-869-8132

 MDCI Ltd.
 Arundel House
 1 Liverpool Gardens
 Worthing, West Sussex BN11 1SL UK



REF

Reorder Information

Catalogue No.	REAG 1	REAG 2
TR81556	1 x 28 mL	1 x 28 mL