CORMAY CRP

DIAGNOSTIC KIT FOR DETERMINATION OF C-REACTIVE PROTEIN CONCENTRATION

Kit name	Kit size	Cat. No
CORMAY CRP	2 x 50 ml + 2 x 10 ml + 1 x 1ml	4-307

INTRODUCTION

CRP (C-reactive protein) is an acute phase protein whose concentration is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infectious, histolytic disease and a variety of disease states. CRP to be used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery. Furthermore, regular measurements of CRP in infants can be a useful aid in the early diagnosis of infectious disease.

METHOD PRINCIPLE

Turbidimetric method, based on C-reactive protein (CRP) reaction with the specific antibodies causing the formation of immune complexes. The increase of absorbance after the addition of antiserum measured at λ=340 nm is proportional to CRP concentration in the sample.

REAGENTS

Package

	CORMAY CRP
1-REAGENT	2 x 50 ml
2-REAGENT	2 x 10 ml
3-STANDARD	1 x 1 ml

Unopened reagents when stored at 2-8°C are stable up to expiry date printed on the package. Do not freeze reagents. Protect from light and avoid contamination!

Concentrations in the test

1-REAGENT

TRIS buffer (pH 7.6)	18.2 mmol/l
sodium chloride	123.2 mmol/l
preservatives	

2-REAGENT

human serum anti-CRP	
TRIS buffer (pH 7.6)	18.2 mmol/l
sodium chloride	123.2 mmol/l
preservatives	

3-STANDARD

Standarized human serum. The exact value of CRP concentration is printed on the label.

Warnings and notes

- Products for in vitro diagnostic use only.
- The reagents must be used only for purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Do not use after expiry date.
- Do not interchange caps.
- After the first opening the 1-REAGENT and 2-REAGENT are stable for 2 months at 2-8°C.
- After the first opening 3-STANDARD, tightly closed is stable for 7 weeks at 2-8°C.
- The reagents contain sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.
- Both 1-REAGENT and 3-STANDARD: clear colourless liquid, 2-REAGENT: pale beige liquid, any significant changes of the colour or turbidity or control sera values outside the manufacturer's acceptable range may indicate instability of the reagents.
- Both 2-REAGENT and 3-STANDARD prepared from human based materials have been tested for the HbsAg and for antibodies to HIV-I, HIV-II and HCV and found to be non-reactive. However this material should be handled as thought capable of transmitting infectious disease.

ADDITIONAL EQUIPMENT

- photometer able to read at 340 nm;
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Nonhemolyzed, nonlipaemic, fresh serum is recommended.
Serum should be separated from morphotic elements within 2 hours after sampling. It is recommended to follow NCCLS procedures regarding specimen collecting and handling.
Serum can be stored for 3 days at 2-8°C or up to 6 months at -20ºC. Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents are dedicated for manual assay.
1-REAGENT and 2-REAGENT are ready to use.
Before use mix all reagents by gently inverting each bottle.

Manual procedure

wavelength	340 nm
temperature	37°C
cuvette	1 cm

3-STANDARD should be diluted with 0.9% NaCl to obtain following serial concentrations:

	S1	S2	S3	S4	S5
Dilution	1/16	1/8	1/4	1/2	neat
Dilution Factor	0.0625	0.125	0.25	0.5	1

Dilutions should be prepared directly before determination.

Pipette into the cuvettes:

	standard (S)	test (T)
1-REAGENT	1000 µl	1000 µl
Sample	-	60 µl
3-STANDARD	60 µl	-

Mix properly, after 5 minutes of incubation at 37°C read absorbance A_1 against air. Then add:

2-REAGENT	200 µl	200 µl

Mix well, after 10 minutes of incubation at 37°C read the absorbance A_2 against air.

Calculation

1. Calculate the change of absorbance for all calibrators:
   $\Delta A = A_2 - A_1$
   And plot the calibration curve. To plot the calibration curve use prepared dilutions only.
2. Calculate the change of absorbance for tested samples. CRP concentrations in tested samples should be read from calibration curve showing the dependence of the absorbance increment ΔA to CRP concentration (mg/l).

REFERENCE VALUES [3, 4]

serum, plasma	
adults	< 0.5 mg/dl (< 5 mg/l)
children (2 months – 15 years)	0.01 – 0.28 mg/dl (0.1 – 2.8 mg/l)
newborns (0 – 3 weeks)	0.01 – 0.41 mg/dl (0.1 – 4.1 mg/l)

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
For internal quality control it is recommended to use the control sera produced by Audit Diagnostics: the CRP Control Level 1 (Cat. No AD924) and CRP Control Level 2 (Cat. No AD934) with each batch of samples.
For the calibration the 3-STANDARD is recommended (enclosed to the set).
The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
These metrological characteristics have been obtained using the automatic analyser Hitachi 911. Results may vary if a different instrument or a manual procedure is used.

- **Detection limit:** 0.5 mg/l.

- **Linearity:** Kit is linear in concentration's range of calibrators. For higher concentrations sample should be dilute with 0.9% NaCl solution, repeat determination and multiple result by dilution factor.

- **Specificity / Interferences**
  Haemoglobin up to 5 g/l, bilirubin up to 600 µmol/l, intralipid up to 5 g/l do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/l]	SD [mg/l]	CV [%]
level 1	11.9	0.40	3.34
level 2	85.3	1.82	2.13

Reproducibility (day to day) n = 20	Mean [mg/l]	SD [mg/l]	CV [%]
level 1	14.9	0.19	1.25
level 2	88.9	1.34	1.51

- **Method comparison**

A comparison between CORMAY kit (y) and another commercially available kit (x) using 50 samples gave following results:
y = 1.011 x - 0.523 mg/l;
R = 0.999 (R – correlation coefficient)

TRACEABILITY
3-STANDARD's values were determined in relation to reference material CRM470.

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. Tillet W. S. et al.: Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus., J. Exp. Med., 52, 561 (1930).
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3. Burtis C.A. Ashwood E.R. Bruns D.E ed. „Tietz Textbook of Clinical Chemistry and Molecular Diagnostics" 4th ed. PA WB Saunders, 2006, p.2263.
4. Schlebusch H, Liappis N, Kalina E, Klein G. High Sensitive CRP and Creatinine: Reference Intervals from Infancyto Childhood. J Lab Med 2002; 26:341-346.

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MANUFACTURER

PZ CORMAY S.A.
22 Wiosenna Street,
05-092 Łomianki, POLAND
tel.: +48 (0) 22 751 79 10
fax: +48 (0) 22 751 79 14
http://www.cormay.pl

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