



Instructions for Use

hs CRP ELISA RUO

RUO

REF EIA-4584R



96



DRG 

DRG Instruments GmbH, Germany
Frauenbergstraße. 18, D-35039 Marburg
Phone: +49 (0)6421-1700 0, Fax: +49 (0)6421-1700 50
Website: www.drg-diagnostics.de
E-mail: drg@drg-diagnostics.de

Distributed by:

DRG 

DRG International, Inc., USA
841 Mountain Ave., Springfield, NJ 07081
Phone: (973) 564-7555, Fax: (973) 564-7556
Website: www.drg-international.com
E-mail: corp@drg-international.com

Please use only the valid version of the Instructions for Use provided with the kit.

Table of Contents

1	PRINCIPLE OF THE CRP ELISA.....	2
2	REAGENTS	2
3	MATERIALS REQUIRED BUT NOT SUPPLIED.....	3
4	WARNINGS AND PRECAUTIONS FOR USERS	3
5	STORAGE CONDITIONS	3
6	SPECIMEN COLLECTION AND PREPARATION.....	3
7	ASSAY PROCEDURE	3
8	RESULTS.....	4
9	EXPECTED VALUES.....	4
10	TEST CHARACTERISTICS	5
11	TEST VALIDITY	5
12	TROUBLE SHOOTING	5
	SYMBOLS USED.....	6

For Research Use Only**Not for use in diagnostic procedures**

Enzyme Immunoassays for the Quantitative High Sensitive Determination of C-Reactive Protein in Human Serum and Plasma.

1 PRINCIPLE OF THE CRP ELISA

Microtiter strips coated with anti-CRP antibody are incubated with diluted standard sera and samples. During this incubation step CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies.

After removal of the unbound conjugate, the strips are incubated with a chromogen solution containing tetramethylbenzidine and hydrogen peroxide: a blue color develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H₂SO₄ and the absorbance values at 450 nm are determined.

A standard curve is obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in samples is determined by interpolation from the standard curve.

2 REAGENTS

- Coated Microtiter strips - [MTP]**
12 x 8-well strips coated with monoclonal antibodies to human CRP.
- Standard Sera - [CAL N]** - 5 vials,
each containing 1/10 prediluted CRP standard solutions (0.2 mL):
N having following values:
CAL 0: 0 µg/mL; CAL 0.4: 0.4 µg/mL; CAL 1: 1 µg/mL; CAL 5: 5 µg/mL; CAL 10: 10 µg/mL.
Contains 0,09 % NaN₃.
Calibrated against the NIBSC 1st International Standard, 85/506.
- Conjugate - [CONJ]** - 1 vial,
containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 mL).
Contains antimicrobial agents and an inert red dye.
- Specimen Dilution Buffer - [DIL 5x]** - 1 vial,
containing 40 mL dilution buffer 5x concentrated.
Contains 0.09 % NaN₃ and an inert green dye.
- Washing Solution - [WASH 20x]** - 1 vial
containing 50 mL 20 x concentrated phosphate buffered washing solution.
- Chromogen Solution - [CHROM]** - 1 vial,
containing 15 mL of a solution containing H₂O₂ and tetramethylbenzidin.
- Stopping Solution - [STOP]** - 1 vial,
containing 12 mL of 0.5M H₂SO₄

Controls can be ordered separately:

[REF] EIA-4584-CTRL

Level 1 & 2; 0.5 mL per level (lyophilized)

3 MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiter plate reader capable of measuring absorbencies at 450 nm

4 WARNINGS AND PRECAUTIONS FOR USERS

1. For research use only.
2. Human blood components used in the preparation of the standard sera have been tested and found to be nonreactive for hepatitis B surface antigen and HIV I. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standard sera in the same way as potentially infectious material. Dispose of samples and all materials used to perform this test as if they contain infectious agents.
3. Do not mix reagents or coated microtiter strips from kits with different lot numbers.
4. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0.3 %. It is classified as a Reproductive Toxicant Category 1B.
Following hazard statements are applicable:
H360D: May damage the unborn child.
Following precautionary statements are applicable:
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P308 + P313: If exposed or concerned: Get medical advice/attention.
5. Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

5 STORAGE CONDITIONS

2 °C to 8 °C

1. Store the microtiter strips in their original package with the desiccant until all the strips have been used.
2. Never use any kit components beyond the expiration date.

6 SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay.

Remove serum from clot as soon as possible to avoid hemolysis. Lipemic and/or hemolyzed samples can cause false results.

Transfer the serum to a clean storage tube.

Specimens may be stored at 2 °C - 8 °C for a few days, or they can be stored frozen for a longer period of time. Avoid repeated freezing and thawing.

7 ASSAY PROCEDURE

○ General Remarks

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. Absorbance is a function of the incubation time and temperature. Therefore the size of the assay run should be limited. It is suggested to run no more than 20 samples with one set of Reference Standards in duplicate.
5. If an ELISA Washer is used, adaptation of the washing step might be necessary to obtain optimal results.

○ Reconstitution of the Reagents

Washing Solution:

Dilute 50 mL of concentrated Washing Solution (5) to 1000 mL with distilled water. Reconstituted solution can be stored at least 1 month, store at 2 °C - 8 °C.

At higher temperatures, the concentrated Washing solution (5) may appear cloudy, without affecting its performance. Upon dilution, the solution will be clear.

Specimen Dilution Buffer:

Dilute 40 mL of the concentrated Specimen Dilution Buffer: to 200 mL with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear. Store at 2 °C - 8 °C.

o Assay Procedure

1. The 10x prediluted standard sera (2) are diluted 1:100 as follows :
pipette 10 µL of each calibrator into separate glass or plastic dilution tubes.
Add 990 µL of diluted Specimen Dilution Buffer and mix carefully.
2. The samples are diluted 1:1000 in two consecutive steps:
pipette 10 µL of each sample into separate glass or plastic dilution tubes and add 990 µL of diluted Specimen Dilution Buffer. Mix thoroughly.
Add 450 µL of diluted Specimen Dilution Buffer to 50 µL of these 100x prediluted samples. Mix thoroughly.
Warning: do not store the diluted samples for more than 8 hours.
3. Pipette 100 µL of the diluted calibrators and samples into each of a pair of adjacent wells (1).
4. Incubate the covered microtiter strips for **30 ± 2 min at room temperature**.
5. Wash the microtiter strips three times with Washing Solution.
This can either be performed with a suitable microtiter plate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2 - 3 minutes. Change washing solution for each cycle. Finally empty the microtiter strips and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add 100 µL of Conjugate Solution (3) and incubate the covered microtiter strips for **30 ± 2 min at room temperature**.
7. Repeat the washing procedure as described in step 5.
8. Add 100 µL of Chromogen (6) Solution to each well.
9. Incubate for **10 ± 2 min at room temperature**. Avoid light exposure during this step.
10. Add 50 µL of Stopping Solution (7) to each well.
11. Determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

8 RESULTS

The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. log/linear) is constructed.

Use the average absorbance of each sample obtained in the hsCRP ELISA to determine the corresponding value by simple interpolation from the curve.

Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.

9 EXPECTED VALUES

Example of typical O.D. values

CALIBRATOR µg/mL	O.D.
0	0.015
0.4	0.112
1	0.354
5	1.370
10	1.954

10 TEST CHARACTERISTICS

10.1 Cross-reactivity

The hs CRP ELISA recognizes natural and recombinant human CRP. No cross-reactivity was observed with following factors, prepared at 1 µg/mL in sample diluent: human pentraxin 2; human pentraxin 3; human monomeric CRP; rat CRP.

10.2 Detection Limit

The minimal detectable concentration is approximately 0.02 µg/mL.

11 TEST VALIDITY

The following specifications must be met for each run to be valid:

O.D. value for the zero calibrator: < 0.080







O.D. value for the highest value calibrator: > 1.000

If one of the specifications is not met, the test run should be repeated.

12 TROUBLE SHOOTING

In case of high background signal, the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

SYMBOLS USED

Symbol	English
	Consult instructions for use
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Temperature limit
	Use-by date
	Manufacturer
	Caution
RUO	For research use only
<i>Distributed by</i>	Distributed by
<i>Content</i>	Content
<i>Volume/No.</i>	Volume / No.

(21-Feb-2020_ia)