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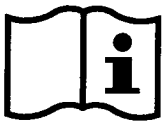

CRP ELISA

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

REF IB79102

Σ 96 wells

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P, Minneapolis, MN 55432
Toll Free: (888) 523-1246 Fax: (763) 780-2988
www.ibl-america.com / info@ibl-america.com

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1. INTENDED USE

The CRP ELISA is an enzyme immunoassay for the determination of C-Reactive Protein in human serum and plasma. For Research Use Only – **Not for Use in Diagnostic Procedure**

2. PRINCIPLE OF THE CRP ELISA

Microtiter strips coated with anti-CRP antibody are incubated with diluted standard sera and patient 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 colour 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 H_2SO_4 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 patient samples is determined by interpolation from the standard curve.

3. REAGENTS

1. **SORB MT Coated Microtiter strips** 12 x 8-well strips coated with monoclonal antibodies to human CRP.
2. **CAL A – EStandard Sera** 5 vials, each containing 1/10 prediluted CRP standard solutions (0.2 ml), N having following values: N=0: 0 µg/ml; N=5: 5 µg/ml; N=25: 25 µg/ml; N=50: 50 µg/ml; N=100: 100 µg/ml. Contains 0,09 % NaN3. Calibrated against the NIBSC 1st International Standard, 85/506 (specification 90-110% agreement).
3. **ENZ CONJ Conjugate** 1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 ml). Contains antimicrobial agents and an inert red dye.
4. **SAM DIL 5x Specimen Dilution Buffer** 1 vial, containing 40 ml dilution buffer 5x concentrated. Contains 0.09 % NaN3 and an inert green dye.
5. **WASH SOLN 20x Washing Solution** 1 vial containing 50 ml 20x concentrated phosphate buffered washing solution.
6. **SUB TMB Chromogen Solution** 1 vial, containing 15 ml of a solution containing H2O2 and tetramethylbenzidine.
7. **STOP SOLN Stopping Solution** 1 vial, containing 12 ml of 0.5M H2SO4.

4. 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 absorbance at 450 nm.

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5. WARNINGS AND PRECAUTIONS FOR USERS

1. For research use only.
2. For professional laboratory use.
3. 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.
4. Dispose of patient samples and all residual products, containers and residues from tests using= these reagents as if contaminated with potentially infectious substances. Safe disposal of residual= products and their containers or packaging and residues from tests using these reagents must be= in accordance with hospital policies and local and/or national legislation.
5. Do not mix reagents or coated microtiter strips from kits with different lot numbers.
6. 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
7. 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.

6. STORAGE CONDITIONS

1. Store the microtiter strips in their original package with the desiccant until all the strips have been used. Opened components should be stored at 2-8°C until next use and can be maintained for 6 months.
2. Never use any kit components beyond the expiration date.

7. SAMPLE COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay. Remove serum from clot as soon as possible to avoid haemolysis. Lipemic and/or haemolysed samples can cause false results. Transfer the serum to a clean storage tube. Specimens may be stored at 2-8 °C for a few days (3 days)[(1)], or they can be stored frozen for a longer period of time (6 months at -20 °C, indefinitely at -70 °C)[(1)]. Avoid repeated freezing and thawing.

8. 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. 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 – 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 (**4**) 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 – 8 °C.

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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 (4) and= mix carefully.
2. The samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient=sample into separate glass or plastic dilution tubes and add 990 µl of diluted Specimen Dilution=Buffer (4). 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 min.= 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 5.
8. Add 100 µl of Chromogen Solution (**6**) 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 or at 450 nm with reference filter 600-650 nm= within 30 min following the addition of acid.

9. RESULTS

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

Precision

Precision has been determined by assaying three spiked samples (ANOVA analysis).
Repeatability (replicate-to-replicate variability) and within laboratory precision (run-to-run and day-to-day variability) has been assessed in a 20 x 1 x 5 study design (20 days, 1 run/day, 5 replicates/run (n = 100 for each sample)), performed by one operator for one batch of the CRP ELISA.

Obtained results:

		Repeatability		Within-Laboratory Precision	
Sample	**Mean (µg/ml)**	**SD (µg/ml)**	**CV (%)**	**SD (µg/ml)**	**CV (%)**
Level 1	3.47	0.271	7.8	0.323	9.3
Level 2	31.51	1.481	4.7	2.962	9.4
Level 3	75.01	4.276	5.7	8.776	11.7

Reproducibility (site-to-site and instrument-to-instrument variability) has been determined based on a 3 x 5 x 1 x 5 study design (3 sites, 5 days, 1 run/day, 5 replicates/run (n = 75 for each sample)) performed by three operators, with three instruments, at three sites for one batch of the CRP ELISA.

Obtained results:

		Reproducibility	
Sample	**Mean (µg/ml)**	**SD (µg/ml)**	**CV (%)**
Level 1	3.54	0.521	14.7
Level 2	33.19	3.983	12.0
Level 3	75.71	11.963	15.8

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10. ANALYTICAL SPECIFICITY

Cross-reactivity
The 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.

11. DETECTION CAPABILITY

The characteristics Limit of Blank (LoB), Limit of Detection (LoD)

Following results are obtained:
LoB: 0,052 µg/ml
LoD: 0,225 µg/ml

12. TEST VALIDITY

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

OD value for the zero calibrator:	< 0.080
OD value for the highest value calibrator:	> 1.000

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

17. 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).

18. REFERENCES

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta

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