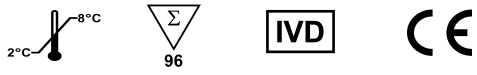
IMMUNOASSAYS AND SERVICES
BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY

LABOR DIAGNOSTIKA NORD GmbH & Co.KG | Am Eichenhain 1 | 48531 Nordhorn | Germany | Tel. +49 5921 8197-0 | Fax +49 5921 8197-222 | info@ldn.de | www.ldn.de

Instructions for use

CRP ELISA

REF **DM E-4600**



CRP ELISA

INTENDED USE

For the quantitative determination of C-Reactive Protein by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of CRP is conjugated to horse radish peroxidase (HRP). CRP from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of CRP in the sample.
A set of standards is used to plot a standard curve from which the amount of CRP in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

C-reactive protein (CRP) is a pentameric acute phase reactant that is synthesized by the liver. Its production is controlled primarily by interleukin-6. The serum CRP concentration may increase by up to 1000-fold with infection, trauma, surgery, and other acute inflammatory events. Chronic inflammatory disorders such as auto-immune diseases and malignancy can produce persistent high levels of serum CRP.
Traditionally, CRP has been used clinically for the diagnosis and monitoring of auto-immune and infectous disorders. Recent studies have shown that chronic inflammation is an important component in the development and progression of atherosclerosis. As a result, increased serum CRP concentration are positively associated with the risk of future coronary events.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A standard curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of CRP in human serum. The kit is not calibrated for the determination of CRP in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only Standard A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical

diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

Dilute patient serum samples 1:20 with Standard A before use.

Example: To 190 µl of Standard A add 10 µl of serum sample (1:20).

⚠ *Do not dilute the standards and controls, they are ready for use.*

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 10, 20, 50, 100, 190, 200 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 13).

REAGENTS PROVIDED

1. AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate –** Requires Preparation **X10**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 ml/bottle
Storage:	Refrigerate at 2 - 8 °C
Stability:	12 months or as indicated on label.
Preparation:	Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

2. AA E-0055 SUBSTRATE **TMB Substrate** - Ready To Use.

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 ml/bottle
Storage:	Refrigerate at 2 - 8 °C
Stability:	12 months or as indicated on label.

3. AA E-0080 STOP-SOLN **Stopping Solution** - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/bottle

Storage: Refrigerate at 2 - 8 °C

Stability: 12 months or as indicated on label.

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

4. Standards and **Controls**- Ready To Use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Standards	Concentration	Volume/Vial
DM E-4601	STANDARD A	**Standard A**	0 ng/ml	16 ml
DM E-4602	STANDARD B	**Standard B**	100 ng/ml	0.5 ml
DM E-4603	STANDARD C	**Standard C**	400 ng/ml	0.5 ml
DM E-4604	STANDARD D	**Standard D**	1000 ng/ml	0.5 ml
DM E-4605	STANDARD E	**Standard E**	4000 ng/ml	0.5 ml
DM E-4606	STANDARD F	**Standard F**	10,000 ng/ml	0.5 ml
DM E-4651	CONTROL 1	**Control 1**	Refer to vial labels for expected value and acceptable range!	0.5 ml
DM E-4652	CONTROL 2	**Control 2**		0.5 ml

Contents: CRP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of CRP. Calibrated against World Health Organization (WHO) 1st IS 85/506.

Storage: Refrigerate at 2 - 8 °C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. DM E-4613 ASSAY-BUFF **Assay Buffer** - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 40 ml/bottle

Storage: Refrigerate at 2 - 8 °C

Stability: 12 months or as indicated on label.

6. DM E-4631 96 **Mouse Anti-CRP Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2 - 8 °C

Stability: 12 months or as indicated on label.

7. DM E-4640 CONJUGATE-CONC **Mouse Anti-CRP Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate –** Requires Preparation **X80**

Contents: Anti-CRP monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.3 ml/vial

Storage: Refrigerate at 2 - 8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:80 in assay buffer before use (eg. 25 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 150 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

ASSAY PROCEDURE

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

⚠	***Dilute serum samples 1:20 with Standard A before use.***
1.	Prepare **working solutions** of the **anti-CRP-HRP conjugate** and **wash buffer.**
2.	Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3.	Pipette **20 µl** of each **standard, control** and **diluted specimen samples** into correspondingly labelled wells in duplicate.
4.	Pipette **200 µl** of **assay buffer** into each well. *(We recommend using a multichannel pipette).*
5.	**Incubate** on a plate shaker (approximately 200 rpm) for **30 minutes** at **room temperature**.
6.	Wash the wells **3 times with 300 µl** of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry *(The use of a washer is recommended).*
7.	Pipette **100 µl** of the **conjugate working solution** into each well. *(We recommend using a multichannel pipette).*
8.	Incubate on a plate shaker (approximately 200 rpm) for **15 minutes** at **room temperature**.
9.	Wash the wells **3 times with 300 µl** of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry *(The use of a washer is recommended).*
10.	Pipette **100 µl** of **TMB substrate** into each well at timed intervals.
11.	Incubate on a plate shaker for **10-15 minutes** at **room temperature**. *(or until Standard F attains dark blue colour for desired OD).*
12.	Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 10.
13.	Read the plate on a microwell plate reader at **450 nm** within 20 minutes after addition of the stopping solution.
⚠	*If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.*

CALCULATIONS

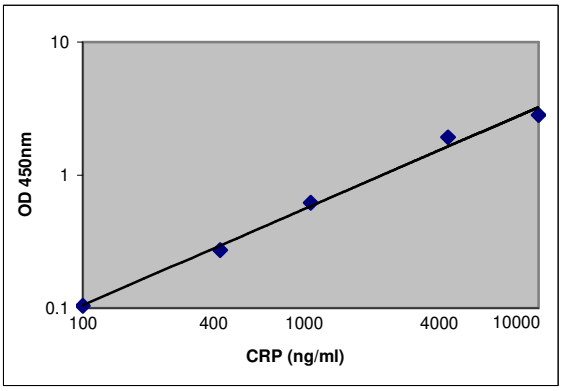
1. Calculate the mean optical density of each standard duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of Standard A from the mean absorbance values of the standards, controls and serum samples.
4. Draw a standard curve on log-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the standard curve.
6. If a sample reads more than 10,000 ng/ml then dilute it with Standard A at a dilution of no more than 1:10 from the original 1:20 diluted serum (or 1:200 from neat serum). The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Standard	OD 1	OD 2	Mean OD	Value (ng/ml)
A	0.055	0.053	0.054	0
B	0.105	0.103	0.104	100
C	0.271	0.276	0.274	400
D	0.607	0.633	0.620	1000
E	1.964	1.894	1.929	4000
F	2.829	2.827	2.828	10,000
Unknown	1.035	1.048	1.042	1737

TYPICAL STANDARD CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Standard A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the hs-CRP ELISA kit is **10 ng/ml.**

SPECIFICITY (CROSS REACTIVITY)

The specificity of the hs-CRP ELISA kit was determined by measuring the apparent CRP value of samples spiked with the following compounds:

Substance	Apparent CRP Value (ng/ml)
Human Albumin	Not Detected
Human Globulin	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same standard curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	205.8	31.2	15.2
2	769.2	38.4	5.0
3	8437.8	700.4	8.3

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	227.0	22.4	9.9
2	1022.2	97.2	9.5
3	8791.8	685.8	7.8

RECOVERY

Spiked samples were prepared by adding defined amounts of CRP to three patient serum samples. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	263	-	-
+358	760	621	122.4
+1430	1820	1693	107.5
+5720	6520	5983	109.0
2 Unspiked	1352	-	-
+358	1880	1710	109.9
+1430	3020	2782	108.6
+5720	7720	7072	109.2
3 Unspiked	5546	-	-
+358	6107	5904	103.4
+1430	6169	6976	88.4
+5720	10400	11266	92.3

LINEARITY
Three patient serum samples were diluted with Standard A. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	3662	-	-
1:5	894	732.4	122.1
1:25	136	146.5	92.8
1:50	62	73.2	84.7
2	6120	-	-
1:4	1922	1530	125.6
1:16	428	382.5	111.9
1:64	110	95.6	115.0
3	8800	-	-
1:4	2472	2200	112.4
1:16	614	550	111.6
1:64	148	137.5	107.6

HIGH DOSE HOOK EFFECT
The hs-CRP ELISA kit did not experience a high dose hook effect when it was tested up to a CRP concentration of 160,000 ng/ml.

EXPECTED NORMAL VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. All values are in ng/ml.

	Males	Females	Combined
N	43	45	88
Age	17 - 87	12 - 79	12 - 87
Abs. Range	73 - 63,680	34 - 39,240	34 - 63,680
2.5th Percentile	132	139	135
50th Percentile	1197	1033	1104
97.5th Percentile	9710	6578	8910

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number		