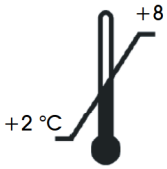
Manual

CRP ELISA Kit

For the in vitro Determination of C-reactive Protein in serum, plasma, stool and urine

Valid from 11.03.2011

K 9710s



1. INTENDED USE

The *Immundiagnostik* Assay is a sandwich Enzyme Immuno Assay intended for the quantitative determination of **C-reactive Protein** in plasma, serum, stool and urine. It is for *in vitro* diagnostic use only.

2. CLINICAL RELEVANCE

C-reactive Protein (CRP) is mainly formed in hepatocytes. The synthesis rate of CRP is influenced by the cytokines involved in the inflammatory processes. The biological half-life time is estimated to be 13-16 hours. The serum CRP concentration reflects very sensitive acute fever, pneumonia and myocardial infraction.

Recent studies describe an association between inflammatory reactions and cardiovascular diseases like arteriosclerosis or latent and chronic persistent infections. As a marker for inflammation, **CRP high-sensitive** can be used to predict the risk of myocardial infarction and stroke.

The CRP determination in urine using the high sensitive ELISA method allows - together with α_2-Macroglobulin - an early screening diagnose after kidney transplantations. The CRP kit provides an easy-to-use assay for monitoring anti-rejection therapies. The ELISA-assay was used for years to test hundreds of patients. Its predictive diagnostic value was compared with the gold standard (kidney biopsy).

Indications

- Prognosis factor for myocardial infarction or stroke
- Inflammatory processes

3. PRINCIPLE OF THE TEST

This Enzyme Immuno Assay is a sandwich assay for the determination of CRP in serum, plasma, urine and stool samples. The wells of the microtitre plate are coated with polyclonal antibodies directed against C-reactive Protein. In a first incubation step, the CRP in the samples is bound to the coated polyclonal rabbit antibodies (in excess). To remove all unbound substances, a washing step is carried out.

In a second incubation step, a Peroxidase-labeled CRP (PO-Antibody, polyclonal, rabbit-anti-CRP) antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stopping solution is then added. The color converts to yellow. The intensity of the yellow color is directly proportional to the concentration of CRP in the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration is generated. CRP, present in the patient samples, is determined directly from this calibration curve.

The combination of two specific antibodies in the CRP ELISA drastically reduces the possibility of wrong-negatives results and offers a secure diagnostic system to the user.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 9710sMTP	PLATE	Micro titre plate with precoated strips	12 x 8 wells
K 9710sWP	WASHBUF	ELISA wash buffer concentrate 10x	1 x 100 ml
K 9710sK	CONJ	POD antibody, (rabbit-anti-CRP, Peroxidase-labeled)	1 x 150 µl
K 9710sST	STD	Calibrators, ready to use (0; 1.9; 5.6; 16.7; 50; 150 ng/ml)	6 x 1 ml
K 9710sKO1	CTRL	Control, ready-to-use	1 x 1 ml
K 9710sKO2	CTRL	Control, ready-to-use	1 x 1 ml
K 9710sPV	SAMPLEBUF	Sample buffer, ready to use	2 x 100 ml
K 9710sTMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 9710sAC	STOP	ELISA stop solution, ready to use	1 x 15 ml

The CRP calibrators were standardized against WHO standard 470.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 10-1000 µl
- Covering foil for the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm

*Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0,2 µm) with an electrical conductivity of 0,055µS/cm at 25°C (≤18,2MΩ cm).

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- The **WASHBUF** (wash buffer concentrate) should be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C using a water bath before dilution. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- The **CONJ** (conjugate) must be diluted **1: 100** in wash buffer (100 µl CONJ + 9900 µl wash buffer). The undiluted conjugate is stable at **2-8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**

- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

7. PRECAUTIONS

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

8. SPECIMEN COLLECTION AND PREPARATION

Serum, plasma

Collection and storage of serum: Collect sufficient blood (at least 1 ml) by venipuncture into a tube or a plastic syringe, avoid hemolysis, centrifuge for 15 minutes at 1,000 x g and 4°C and collect the serum.

Collection and storage of plasma: Collect sufficient blood (at least 1 ml) by venipuncture into an EDTA venipuncture tube or a plastic syringe, centrifuge for 15 minutes at 1,000 x g and 4°C within 10 minutes after blood collection and separate the plasma from the cells.

Serum and plasma samples have to be diluted 1:100 or 1:500 before performing the assay.

Add **10 µl** serum /plasma to **990 µl** dilution buffer, mix well. (1:100)
Use the dilution factor (100 or 500) to calculate the CRP concentration read off the calibration curve.

Patient's samples with elevated CRP-concentrations must be diluted 1:4000 – 1:8000. Samples of other patient collectives must be diluted according to the expected CRP-concentration. The corresponding dilution factor must be used for calculation of the CRP-concentration.

Faeces

The test can be performed on either fresh or frozen stool samples. The samples should be refrigerated and can be stored at 2-8°C for 2 days. If the test cannot be performed within this period, the specimen should be stored at –20°C or colder.

Add a stool sample of about **100 mg** (size of a pea, please note the exact weight for the calculation) to **5 ml** of the ELISA wash buffer and homogenize very thoroughly for 15 seconds on a Vortex-mixer. Centrifuge the suspension for 10 min at 3000 rpm. Pipet 1 ml of the supernatant into an Eppendorf tube and centrifuge at 13,000 rpm for **2 min**. The supernatant can be stored at -20°C for about 1 month. **100 µl** of this supernatant is used in the assay.

Immundiagnostik recommends for sample preparation the use of Roche Diagnostics / Mannheim sample preparation tubes, article No. 745804.

Urine

Urine samples must be diluted **1:5** with dilution buffer.

9. ASSAY PROCEDURE

Procedural notes

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.

Test procedure

1.	Prior to use in the assay allow all **reagents and samples** to come to **room temperature** (18-26 °C) and mix well
2.	Mark the **positions of STD** (Standard) **SAMPLE** (Sample) **CTRL** (Controls) on a protocol sheet
3.	**Take microtiter strips** out of the kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label
4.	Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
5.	Add **100 µl of STD** (Standard) **SAMPLE** (Sample) **CTRL** (Controls) in duplicate into respective well
6.	Cover the plate tightly and incubate for **1 hour at room temperature** (18-26°C) on a horizontal mixer

7.	Discard the contents of each well. Wash **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
8.	Add **100 µl CONJ** (conjugate) into each well
9.	Cover the plate tightly and incubate for **1 hour at room temperature** (18-26°C) on a horizontal mixer
10.	Discard the contents of each well. Wash **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
11.	Add **100 µl of SUB** (substrate) into each well
12.	Incubate for **10 - 20 minutes at room temperature** (18-26°C) in the dark*
13.	Add **50 µl of STOP** (stop solution) into each well, mix thoroughly
14.	Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used

*The intensity of the colour change is temperature sensitive. We recommend to observe the colour change and to stop the reaction upon good differentiation.

10. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point-calculation

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Faecal specimen

Example for calculation of the CRP concentration in faecal specimen

Weight:	80 mg (1ml stool = 1g) = 0.08 ml
Dilution step 1:	5 ml / 0,08 ml = 62.5
Dilution factor:	62.5

Multiply the results with the calculated dilution factor (in this case 62.5) to get the CRP concentration of the stool samples. **Please note:** the dilution factor depends on the weight of the used faecal specimen.

Serum, plasma

The value read from the calibration curve must be multiplied by **100** or **500** respectively to get the CRP concentration in serum/plasma samples.

If samples were diluted 1:4000 or 1:8000, the estimated values must be multiplied by 4000 or 8000 respectively.

Urine

The measured CRP concentration must be multiplied by factor **5** to get the actual concentration of the samples.

11. LIMITATIONS

Samples with CRP levels greater than the highest calibrator value should be diluted and re-assayed.

12. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Normal ranges

CRP-Concentration, **Serum/plasma:**

	< 1 mg/l low CHD-Risk
	1-3 mg/l medium CHD-Risk
	> 3 mg/l high CHD-Risk

*(Pearson et al., 2003)

CRP-Concentration, **Stool**:	< 56 ng/ml
CRP-Concentration, **Urine**:	< 6 ng/ml

If the CRP-concentration is found to be higher than 3 mg/l, a second determination should be made within 2 to 3 weeks. If the CRP-concentration is again high, and other reasons are excluded (acute infection, chronic-inflammatory diseases), the obtained CRP-concentration can be used for risk stratification in coronary heart disease (CHD) patients. If the CHD risk is high, the lifestyle should be changed together with medical treatment.These normal ranges should be used as a guideline only.

It is recommended that each laboratory establishes an own expected range for its patient population.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) of the Immundiagnostik CRP ELISA test was calculated from 20 replicate determinations on each one of two samples.

Intra-Assay CV

n= 20

Sample	CRP Mean value [ng/ml]	Intra-Assay CV [%]
1	23.3	6
2	99.4	5.5

The total precision (inter-assay variation) of the Immundiagnostik CRP ELISA test was calculated from data on 2 samples obtained in 15 different assays by three technicians on two different lots of reagents over a period of three months.

Inter-Assay

CV n= 15

Sample	CRP Mean value [ng/ml]	Inter-Assay CV [%]
1	22.1	11.6
2	90.4	13.8

Recovery

Two samples with known CRP concentration were spiked with four different amounts of CRP and measured.

Recovery n=4

Sample [ng/ml]	Spike [ng/ml]	CRP expected [ng/ml]	CRP measured [ng/ml]
9.8	37.5	47.3	44.5
9.8	18.8	28.6	27.3
9.8	9.4	19.2	18.2
9.8	4.7	14.5	14.3
9.3	37.5	46.8	48.2
9.3	18.8	28.1	26.3
9.3	9.4	18.7	18.0
9.3	4.7	14.0	13.7

Sensitivity

The sensitivity was set as B_0 + 2 SD. The zero-standard was measured 18 times.

n=18

Sample	CRP Mean value [OD]	Standard variation	Detection limit [ng/ml]
1	0.005	0.001	0.921

Linearity

Two patient samples were diluted and analyzed. The results are shown below:

n= 2

Sample	Dilution	Expected [µg/ml]	Measured [µg/ml]	Recovery [%]
A	1:100	2.90	2.88	99.3
	1:200	1.45	1.55	106.8
	1:400	0.73	0.83	113.7
	1:800	0.36	0.39	108.3
	1:1600	0.18	0.18	100.0
B	1:200	10.80	10.80	100.0
	1:400	5.40	5.80	107.4
	1:800	2.70	2.90	107.4
	1:1600	1.35	1.61	119.3
	1:3200	0.68	0.83	122.1
	1:6400	0.33	0.35	106.1

Cross reactivity

Alpha-1-Antitrypsin	0 %
Lysozym	0 %
Albumin	0 %
Other acute phase proteins	0 %

No cross reactivity with CRP in mouse serum was observed.

14. REFERENCES

1. Koenig W et al. (2004) Circulation 109: 1349-1353
2. Pearson TA et al. (2003) Circulation 107 : 645-651
3. Ridker P et al. (2000) N Engl J Med 342: 836-843

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG along with a written complaint.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

Contains sufficient for <n> tests


Manufacturer


Use by

Lot number