

Test Instructions IMTEC-hs-CRP

Enzyme Immunoassay for quantitative high sensitive Determination of C-reactive Protein

REF : TC 59600

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

As a diagnostic marker of acute phase reaction, the fundamental importance for determination of C-reactive protein (CRP) is indisputable, although alternative acute phase markers were described numerously in the last few years. Acute phase reactions are not only triggered by infections, tissue damage, tumor diseases, after surgical intervention but also in myocardial infarction. In rheumatology the determination of CRP as a marker of rheumatoid arthritis activity has gained acceptance in contrast to the unreliable and faulty method of erythrocyte sedimentation rate. However, in systemic lupus erythematosus CRP does not correlate with the disease activity, but enhanced CRP points to intercurrent infections.

Indications for CRP determination are:

- diagnosis and monitoring of infections
- differential diagnosis of bacterial and viral infection
- diagnosis of neonatal infections
- activity determination of rheumatic diseases
- differential diagnosis of gastrointestinal diseases
- indication of rejection crisis after transplantation
- myocardial infarction
- risk judgment for diabetes, coronary heart disease, unstable angina pectoris or haemodialysis.

The diagnostic relevance of CRP can be basically put down to its dynamics regarding velocity and extent of change of concentration. Approximately 6 hours after tissue damage CRP-levels are rising rapidly and decreasing rapidly too after the removal of damaging noxes. The degradation of CRP takes place according to its biological half life time without diurnal fluctuations. Whereas the reference range for adults as well as children is about 1-5 mg/L, during inflammation it can rise to the 1000-fold.

Several prospective studies within the last few years pointed out impressively, that the level of the CRP concentration within the normal reference range is a marker of arteriosclerotic events and a risk parameter for myocardial infarction as well as heart attack in apparently healthy people too. In case of the diagnosis of neonatal infections it was recently shown, that a high sensitive CRP assay is of importance too, since newborn do not produce sufficient amount of CRP and therefore show a less distinctive CRP increase during infection.

2. Principle of the Test

The test is based on the adsorptive immobilisation of anti-CRP antibodies to a solid phase (polystyrene) and subsequent binding of human CRP from serum. CRP is detected with a peroxidase-labeled secondary antibody that is directed against human CRP respectively (sandwich ELISA). After addition of substrate solution, a color stain develops. Its intensity is proportional to the concentration and/or the avidity of CRP. **The testkit contains one set of standards for determination of high CRP concentrations (CAL NS : 18.5-500mg/L) and one for low concentrations (CAL HS : 1.0-27.0 mg/L) covering the normal reference range of CRP.**

3. Materials Provided

-	MTP	: anti-CRP coated microtiter strips (1x8), breakable	12 strips
-	CAL HS	: standards, high sensitive	750 µl per vial
	1	1.0 mg/L CRP	1 vial each
	2	3.0 mg/L CRP	
	3	9.0 mg/L CRP	
	4	27 mg/L CRP	
-	CAL NS	: standards, normal-sensitive	750 µl per vial
	1	18.5 mg/L CRP	1 vial each
	2	55.6 mg/L CRP	
	3	166.7 mg/L CRP	
	4	500 mg/L CRP	
	all standards contain sodium azide, are inked according to concentration and ready to use		
-	CONTROL NS +	: positive control serum for use with CAL NS , ready to use, contains sodium azide	1 vial 1 ml
-	CONTROL HS +	: positive control serum for use with CAL HS , ready to use, contains sodium azide	1 vial 1 ml
-	BUF WASH 10x	: washing buffer concentrate (10x)	1 bottle 50 ml
-	DIL SPE 5x	: sample buffer concentrate (5x)	1 bottle 50 ml
-	CONJ	[a(hum CRP):HRP] : HRP-Conjugate, anti-human CRP, ready to use	1 bottle 12 ml

-	SUBS TMB	: TMB solution, HRP substrate, ready to use	1 bottle 12 ml
-	SOLN STOP	: stopping solution, ready to use, contains sulfuric acid, caution corrosive!	1 bottle 12 ml

4. Preparation of Reagents

Attention!

Allow the testkit and all its components to reach room temperature completely before executing it !

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate **BUF** **WASH** **10x** with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

4.2. Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part sample buffer concentrate **DIL** **SPE** **5x** with 4 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

4.3. Standards, Control Sera, HRP Conjugate, Stopping Solution and TMB Solution

Standards, control sera, HRP Conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.4. Preparation of Serum

Use sera freshly collected or freeze samples at -20°C. Do not use samples, that are repeatedly thawed and frozen. Allow the samples to reach room temperature (30 min).

Dilute sera two-step according to the concentration range expected:

- 1:1.000 for high sensitive CRP (**HS**): 1.0-27.0 mg/L), add 10 µl serum to 1 ml sample buffer (first step), add 100 µl of first dilution to 0,9 ml sample buffer (second step).
- 1:10.000 for normal sensitive CRP (**NS**): 18.5-500mg/L), add 10 µl serum to 1 ml sample buffer (first step), add 10 µl of first dilution to 1 ml sample buffer (second step).

4.5. Microtiter Strips

The strips are ready to use. Unused strips should be sealed in the lockable original bag at 2-8°C.

5. Test Procedure

- Pipette 100 µl serum or standards **CAL** (inked according to rising concentration) or control sera **CONTROL** **+** into each well.
- Use **CONTROL** **NS** **+** together with standards **CAL** **NS** and **CONTROL** **HS** **+** together with standards **CAL** **HS** respectively.
- for blanks use sample buffer instead of serum dilution, seal wells with adhesive foil.

- Incubate for 1 hour at room temperature (RT).
- Rinse the wells 3 x using at least 200 µl washing buffer per well.
- Discard buffer and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl of HRP-conjugate **CONJ** **a(hum CRP):HRP** into each well, seal wells with adhesive foil.
- Incubate for 30 minutes at RT.
- Rinse the wells 3 x using at least 200 µl washing buffer per well.
- Discard buffer and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl TMB solution **SUBS** **TMB** into each well.
- Incubate for 10 min at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- Pipette 100 µl stopping solution **SOLN** **STOP** per well.
- Measure at 450 nm within the next 30 min after stopping.

6. Interpretation of Results

Calibrate measured absorbance against concentration of standards **CAL** **HS** (1.0 mg/L, 3.0 mg/L, 9.0 mg/L, 27.0 mg/L) or standards **CAL** **NS** (18.5 mg/L, 55.6 mg/L, 166.7 mg/L, 500.0 mg/L) in semi log. Determine the concentration of the examined sera samples from the standard curve directly.

To prove the functionality of the test, the determined value for the positive control **CONTROL** **NS** **+** and **CONTROL** **HS** **+** is to be expected within the range quoted on the vial.

reference range: < 5 mg/L

Precautions

For in vitro diagnostics only.

IVD

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10

D-13125 Berlin

GERMANY

Tel.: +49 (30) 94 89 36 00

Fax: +49 (30) 94 89 36 15

www.imtec-immundiagnostika.de

imtec@mdc-berlin.de