invitron
high sensitivity diagnostics

IV3-105E

English

High Sensitivity CRP (hsCRP) ELISA Kit

For in-vitro diagnostic use only

Definitions

	Instructions for use
REF	Catalogue number
	Use by
LOT	Lot/Batch Code
	Storage temperature limitations
IVD	In vitro diagnostic medical device
	Contains sufficient for <N> tests

CE

Invitron Ltd.
Wyastone Business Park, Wyastone Leys
Monmouth NP25 3SR, UK
www.invitron.com

High Sensitivity CRP (hsCRP) ELISA Kit

Intended Use

The hsCRP ELISA Kit is an ELISA for the quantitative measurement of C-Reactive Protein in human serum, plasma or urine samples. It is for *in vitro* diagnostic use only.

Summary and Explanation

C-reactive Protein (CRP) is synthesised mainly by hepatocytes. Its rate of synthesis is regulated by cytokines involved in the inflammatory process and its biological half-life is estimated to be 13-16 hours. Serum concentrations of CRP are elevated by acute fever, pneumonia and myocardial infarction.

Recent studies have established an association between inflammatory reactions and cardiovascular diseases such as atherosclerosis or latent and chronic infections. Thus CRP levels can be used to predict the risk of myocardial infarction and stroke.

In urine, CRP measurement, along with that of α_2-macroglobulin, can be used as an early marker of tissue rejection in patients who have undergone renal transplantation. Thus CRP measurement can provide a simple way of monitoring the efficacy of anti-rejection therapy.

Indications

- Prognostic marker for myocardial infarction or stroke
- Inflammatory disease

Principle

The hsCRP ELISA is a two-site assay for the determination of CRP in serum, plasma or urine samples. The wells of the microtitre plate are coated with rabbit polyclonal antibodies raised against CRP. Samples and standards are incubated in the wells in order to capture CRP. After washing to remove all unbound material a peroxidise-labelled polyclonal antibody is added and a further incubation is carried out. After another wash step, the peroxidise substrate tetramethylbenzidine (TMB) is added and the colour allowed to develop. The reaction is terminated by addition of acid and the absorbance read at a wavelength of 450 nm. Absorbance is proportional to the concentration of CRP in the sample and results are calculated by reference to a calibration curve generated from standard concentrations.

Materials Provided

- Coated Microtitre Plate [PLATE]
  (12 x 8) stripwells coated with a polyclonal antibody to CRP.
- HRP Conjugate [CONJ]
  (1 x 150 µl) peroxidise-labelled rabbit anti-CRP antibody.
- Sample Buffer [SAMPLEBUF]
  (2 x 100 ml) Buffer for dilution of samples, ready to use.
- Standards [STD]
  (6 x 1 ml) Calibrators 0; 1.9; 5.6; 16.7; 50; 150 ng/ml, ready to use. The calibrators are standardised against certified reference material (CRM 470).
- Controls [CTRL]
  (2 x 1 ml) low and high controls, ready to use.
- Substrate [SUB]
  Tertramethylbenzidine (TMB) substrate, supplied ready to use.
- Stop Solution [STOP]
  ELISA Stop Solution, supplied ready to use.
- Wash Buffer Concentrate [WASHBUF]
  Phosphate buffer containing detergent and preservative (10x concentration).

Materials Required but not Provided

- Deionised water
- Precision pipettes and tips able to deliver 10-1000 µl
- Microtitre Plate sealers
- Vortex mixer
- Microtitre plate shaker
- Microtitre plate reader

Warnings and Precautions

- Human materials used in the kit components have tested negative for HIV, hepatitis B and hepatitis C. Nevertheless, for safety reasons, all components should be treated as potentially infective.
- Stop solution is diluted sulphuric acid and therefore must be handled with care. It can cause burns and so gloves, safety glasses and appropriate protective clothing should be worn when handling it. Any spills should be wiped up immediately using large volumes of water.
- Kit reagents contain thiomersal which is toxic. The substrate for the enzyme (TMB) is carcinogenic. Care should be taken to avoid any skin contact with these reagents.
- Reagents should not be used beyond the expiry date shown on the kit label.

Preparation and Storage of Reagents

- Up to 4 runs can be carried out with each kit. **Prepare only the required quantity of reagents for each run.** Ensure that the reagents are stored under the conditions stated on the kit label.
- It is recommended that the small volume of labelled antibody concentrate is centrifuged before use.
- Wash buffer concentrate should be diluted 1:10 in deionised water before use. If any crystals have formed during storage, these should be re-dissolved by warming the wash buffer to 37º prior to dilution. Wash buffer concentrate is stable when stored at 2-8º up to the expiry date on the label. Diluted wash buffer can be stored in a closed vessel for up to 4 weeks at 2-8º.
- Labelled antibody concentrate should be diluted 1:100 in wash buffer before use. The concentrate is stable when stored at 2-8º up to the expiry date**. Dilute labelled antibody is not stable and cannot be stored for further use.**
- All other test reagents are ready to use. These reagents are stable until the expiry date given on the kit label when stored at 2-8º.

Specimen Collection and Preparation

Serum: Whole blood should be taken into a plain tube and allowed to clot for 30 minutes. The clot should be separated by centrifugation. Care should be taken to avoid haemolysis.

Plasma: Whole blood should be collected into a tube containing EDTA anticoagulant and centrifuged immediately after collection.

Before performing the assay, it is necessary to dilute serum or plasma 1:100 or 1:500 with Sample Diluent. This factor must then be taken into account when calculating results from the calibration curve.

Patient samples containing elevated CRP concentrations may need to be diluted by a factor of 1:4000 to 1:8000.

Urine: Urine samples must be diluted 1:5 with Sample Diluent prior to assay.

Assay Procedure

1. Bring all kit components and samples to room temperature before use and mix well.
2. Produce a protocol sheet recording the position of standards, controls and samples.
3. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored covered at 2-8ºC. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
4. Wash each well 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
5. Add 100 µl of standard, control and sample to the respective wells in duplicate.
6. Cover the plate with a plate sealer and incubate for 1 hour at room temperature on a plate shaker.
7. Discard the contents of the wells. Wash 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
8. Add 100 µl of diluted labelled antibody into each well.
9. Cover the plate with the sealer and incubate for 1 hour at room temperature on a plate shaker.
10. Discard the contents of the wells. Wash 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
11. Add 100 µl of substrate into each well.
12. Incubate for 10-20 minutes at room temperature in the dark.*
13. Add 50 µl of stop solution into each well.
14. Read the absorption in an ELISA reader at a wavelength of 450 nm. Ideally, results should be compared with extinctions measured at a reference wavelength (eg 620 nm). If the extinction obtained for Standard 6 falls outside the range of the photometer, the plate should be read immediately at 405 nm and the results so obtained used for calculation purposes.

*The rate of colour change is temperature dependent. It is recommended that the development reaction is stopped when the colour in the standards shows good discrimination.

Results

The results may be calculated automatically using a cubic spline or 4-parameter curve fit. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard should be further diluted**. For the calculation of the concentrations in serum/plasma or urine samples, the appropriate dilution factor must be taken into account.**

Expected Values

If the CRP concentration in serum/plasma is greater than 3 µg/ml, it is recommended that a second determination is performed within 2-3 weeks. If the CRP concentration continues to be elevated and other causes (acute infection, chronic inflammatory disease) are excluded, the results can be used for risk stratification for coronary heart disease (CHD). **These ranges should be used only as a guideline.** It is recommended that each laboratory establishes its own expected ranges for its patient population.

CRP Concentrations in serum/plasma (Ridker PM, 2003)	<1 µg/ml – low CHD risk 1-3 µg/ml – medium CHD risk >3 µg/ml – high CHD risk
CRP concentrations in urine	<6 ng/ml

Limitations

- Only if test instructions are rigidly followed will optimum results be achieved.
- Use fresh plasma or specimens frozen and thawed no more than twice.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. If, after checking the above mentioned items, there is no error, contact Invitron directly.

Performance

Precision

Intra-assay precision was calculated from the results obtained for 20 replicate determinations at two dose levels.

Intra-Assay CV

Sample	Mean CRP (ng/ml)	CV (%)
1	23.3	6.0
2	99.4	5.5

Inter-assay precision was calculated from data obtained for 2 samples measured in 15 assays over a period of 3 months.

Inter-Assay CV

Sample	Mean CRP (ng/ml)	CV (%)
1	22.1	11.6
2	90.4	13.8

Recovery

Two samples were spiked with four different amounts of CRP and then assayed in the hsCRP ELISA. The following table compares the measured results with those expected from the spiked samples.

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

Linearity

Two patient samples were diluted and measured in the Invitron CRP ELISA. The results are shown in the following table.

Sample	Dilution	CRP Expected (µg/ml)	CRP 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

References

Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003, 107: 363-369.

For additional information and product support please contact:

Invitron Limited
Wyastone Business Park
Wyastone Leys
Monmouth NP25 3SR
UK
Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
info@invitron.com
www.invitron.com