Rabbit CRP ELISA KIT

Research Reagent

For in-vitro laboratory use only

Please, read this instruction carefully before use.
This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of Rabbit CRP (C-Reactive protein) with high sensitivity using Sandwich assay principle.

Advantage

(1) Rapid assay (total reaction time: 2 hours 30min.).
(2) A small sample volume (50µl).
(3) An ecologically excellent preservative is used.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.

Components

	Reagents	Amounts
(A)	Anti-CRP-coated plate	96 wells(8x12) / 1 plate
(B)	Standard CRP solution (2µg/ml)	200µl / 1 vial
(C)	Buffer solution	60ml/1 vial
(D)	Peroxidase-conjugated anti-CRP antibody	200µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 vial
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 vial
(I)	Concentrated washing buffer (10x)	100ml/ 1 bottle

Assay samples

Rabbit serum or plasma diluted properly with buffer (C).
For normal rabbit serum, 100X dilution would be proper.

Assay range

3.13 ~ 200ng/ml

Assay operation

1. Equipments necessary but not included in the kit.
   (1) Micropipette (a micropipette able to deliver sample volume with high precision.), and a pipette for repeating delivery.
   (2) Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
   (3) A microplate reader (A densitometer for microplate).

2. Preparation of reagents
   (1) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.
   (2) Peroxidase-conjugated anti-CRP (D) : Dilute to 100X with the buffer solution(C).
   (3) Other reagents are used as they are.
   (4) All the reagent solutions should be used after getting back to room temperature (20-25C).

3. An example of preparing standard solutions
   Dilute the original standard solution (B) with the buffer solution to prepare 200ng/ml,then prepare lower standard solutions by a dilution program shown below.

Concentration,ng/ml	200	100	50	25	12.5	6.25	3.13	0
Standard. Solution,µl	50 (orig.sol.)	200*	200*	200*	200*	200*	200*	0
Buffer,µl	450	200	200	200	200	200	200	200

*One rank higher standard solution

4. Assay procedure
   Remove the cover sheet of the microplate after getting back to room temperature.

(1) Rinse the anti-CRP coated wells (A) by filling the washing buffer and discard 3 times, then strike the plate upside-down onto folded several sheets of paper towel, and remove the excess buffer.
(2) Pipette 50µl of sample solution to the wells for samples.
(3) Pipette 50µl of the standard solution to the wells for preparing standard curve.
(4) Shake the plate gently on a plate shaker for 10-15 seconds.
(5) Incubate for 1 hour at room temperature (20-25C).
(6) Discard the reaction mixture, and then wash wells as described in (1).
(7) Pipette 50µl of peroxidase-conjugated anti-CRP solution to all wells. Then shake gently on a plate shaker for 10-15 seconds.
(8) Incubate the plate for 1 hour at room temperature.
(9) Discard the reaction mixture, and then wash the plate as (1).
(10) Pipette 50µl of chromogenic substrate solution to wells, and shake as (4).
(11) Let the plate stand for 30 minutes at room temperature.
(12) Add 50 µl of the reaction stopper (H) to all wells and shake.
(13) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

Summary of Assay Procedure

Antibody-coated 96 well plate
↓
Washing 3 times
↓
Sample or Standard 50µl
↓
Shaking and reaction for 1 hour at room temp.
↓
Washing 3 times
↓
Peroxidase-conjugated anti-CRP 50µl
↓
Shaking, and reaction for 1 hr. at room temp.
↓
Washing 3 times.
↓
Chromogenic substrate solution 50µl
↓
Shaking, and reaction for 30 mins. at room temp
↓
Reaction stopper 1M H_2SO_4) 50µl
↓
Shaking and measurement of absorbance at 450nm(sub. 620nm)

Room temp.: 20~25C

Calculation of CRP concentration

(1) Prepare a standard curve using semi-logarithmic section paper by plotting absorbance* (Y-axis) against CRP concentration (ng/ml) on X-axis.
*Absorbance at 450nm minus absorbance at 620nm.
(2) Using the standard curve, read the CRP concentration of a sample from its absorbance*, and multiply the assay value by dilution rate if the sample has been diluted. Though the assay range is wide enough, in case the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.
* We recommend the use of 3rd order regression curve or 4 parameter method in computer calculation.

Important notice in the treatments

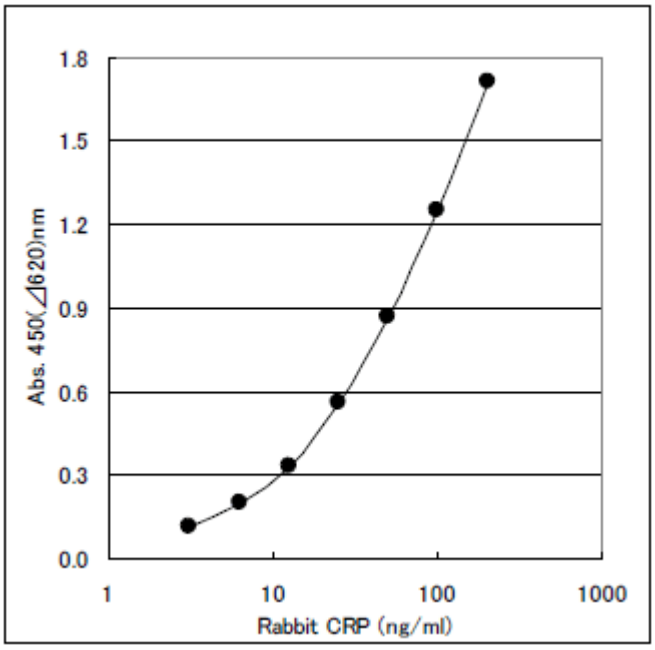
1. Treatment of assay samples
(1) Use serum or plasma samples obtained by ordinary standard method.
Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.
(2) Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
(3) Measure the samples as soon as possible after sampling.

2. Storage of assay samples.
If assay samples have to be stored for a long period, freeze samples and store below .35C. Avoid repeated freezing and thawing.

3. Influence of interfering substances
If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

Assay range and assay validation

1. A model standard curve



2. Specificity

Cross-reaction to other rabbit serum protein is less than lower detection limit.ris Antibodies GmbH/PA1199

3. Precision and reproducibility

(1) Within assay variation (3 samples, 8 replicates assay)

Average C.V. is 3.6%.

(2) Reproducibility (3 samples, duplicates assay, 3 days)

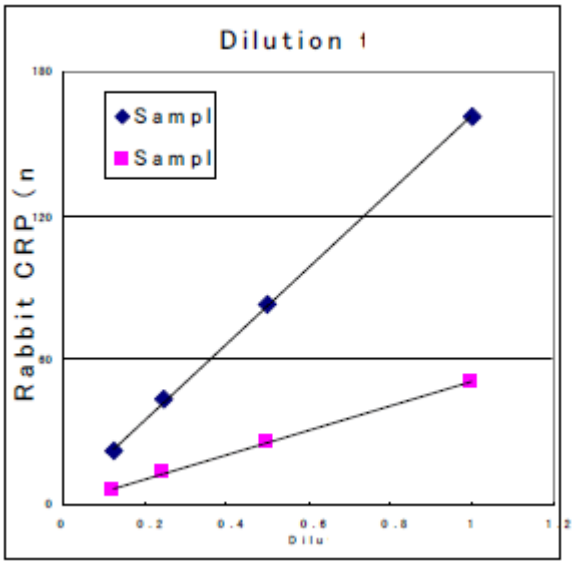
Average C.V. is less than 5%.

4. Recovery test

Added	Found	Recovered	Recovery (%)
0	4.73	-	-
2.98	7.86	3.13	105
4.47	9.35	4.62	103
5.95	10.7	5.97	100
7.44	12.4	7.67	103
Added	Found	Recovered	Recovery (%)
0	19.6	-	-
18.2	38.2	18.6	102
36.3	58.2	38.6	106
54.5	75.0	55.4	102
72.6	98.1	78.5	108

ng/ml, n=2

5. Dilution test



6. Serum CRP levels of normal rabbits
Samples: normal Japanese white rabbits, 24 . 30 months of age, females
Free access to food and water. n=22
Mean assay value: 6.07 µg/ml SD: 2.93 µg/ml

Statements and precaution

1 The reagents included in this assay kit should be used only for research works.
2 The reagent solutions of the kit should be used principally immediately after reconstitution. Otherwise, keep them in a dark place with the temperature 2-8Cï¼Œand use them within 3 days.
3 The reagents were prepared to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number. Even the lot number is the same, do not mix the reagents with those that have been preserved for some period.
4 Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
5 Do not dry the assay plate to avoid denaturation of the coated antibody.
6 Measurement of the reaction time should be started from the pipetting of reagent to the first well.
7 Prepare the standard curve in each assay.
8 Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
9 Storage condition for the kit should be strictly followed.
10 Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Especially be careful for the stopping solution because it is 1M sulfuric acid.
11 HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper must be avoided from contacting with any metal.
12 In treating assay samples of animal origin, be careful for possible biohazards.
13 As the antibody-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

Storage condition

Store the kit at 2~8C. Do not freeze.

Term of validity

Six months from production. Expiration date is indicated on the container.

Unit of package

96-wells/1 plate

Product code

AKRCR-017

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