

File S2. Methods of a reagent preparation.

1) Preparations for Anti-human CRP (Anti-h CRP 6407 SPTN-5)-sensitized colloidal gold and Streptoavidin-sensitized colloidal gold.

- a) Anti-human CRP was adjusted to 100 µg/ml after substitution with phosphate buffer.
- b) Add 100 µl of potassium dihydrogen phosphate buffer to a 2.0 ml tube.
- c) 900 µl of colloidal gold solution was added to the above tube and gently stirred.
- d) Add 100 µl of the antibody solution (100 µg/ml) prepared in a) to the above tube while stirring, and incubate at room temperature for 10 min,
- e) 55 µl of polyethylene glycol solution was added to the above tube and gently stirred.
- f) 110 µl of BSA solution was added to the above tube and gently stirred.
- g) The tube was centrifuged at 8,000g for 15 min at 4°C.
- h) The supernatant was removed except for about 100 µl, and the precipitate was dispersed in the remaining supernatant for 2 min.
- i) 1.5 µl of the dispersed sensitized colloidal gold solution was taken and the optical density at 520 nm was measured.
- j) The optical density at 520 nm was adjusted to 6.0 with colloidal gold storage buffer.
- k) The reagent was stored at 4°C until its use.

Streptoavidin was dissolved in ultrapure water to a concentration of 100 µg/ml, and the same procedure was performed.

2) Fabrication of conjugate pad.

- a) The anti-human CRP (Anti-h CRP 6407 SPTN-5)-sensitized colloidal gold solution and the streptoavidin-sensitized colloidal gold were separated using an ultrasonic cleaner.
- b) Each colloidal gold solution, ultrapure water, and gold colloid coating buffer were mixed in a 2 ml tube.
- c) The entire amount was applied evenly onto a glass fiber pad.
- d) The pad was placed in a desiccator and dried under reduced pressure for more than one day.
- e) The pad was stored in a refrigerator until its use.

3) Preparation of biotinylated BSA.

- a) BSA was dissolved to 5 mg/ml with carbonate buffer so that the final volume was 1,000 µl.
- b) The biotinylation reagent was adjusted to 40 mg/ml with ultrapure water so that the final volume was at least 12.5 µl.
- c) 12.5 µl of the biotin reagent solution prepared in b) was added to 1,000 µl of the BSA

solution prepared in a).

- d) The mixed solution was allowed to stand at room temperature for 2 h.
- e) Glycine was adjusted to 10 mg/ml with ultrapure water so that the final liquid volume was 100 µl or more.
- f) 100 µl of the glycine solution prepared in e) was added to the tube in d).
- g) The solution was replaced with PBS.
- h) After measuring the optical density at 280 nm of the internal solution, it was adjusted with PBS to 1 mg/ml and stored in a refrigerator until its use.

4) Preparation of antibody-immobilized membrane.

- a) Anti-human CRP antibody (Goat anti-human CRP antibody) was replaced with PBS and adjusted to two different concentrations.
- b) 100 µl of the anti-CRP polyclonal antibody as test line 1 and 2, and the biotin-labelled BSA (1.0 mg/ml) as a control line were prepared.
- c) The antibodies were applied to a membrane using a coating machine.
- d) The membrane was dried at 37°C for 1 h.
- e) The entire membrane was immersed in blocking buffer, and it was allowed to stand at room temperature for 30 min.
- f) The membrane was transferred to the washing/stabilizing buffer for 30 min at room temperature while stirring.
- g) It was dried at room temperature for more than one day and night.
- h) It was stored in a refrigerator protected from the light.

5) Assembly of immunochromato strip.

- a) The antibody-immobilized membrane was placed at a position of 20 mm from the bottom edge of the backing sheet.
- b) The sample absorbent pad was aligned with the top edge of the backing sheet and pasted.
- c) A plasma separation pad was attached at 17 mm from the bottom edge of the backing sheet.
- d) A conjugate pad was pasted 11 mm from the bottom edge of the backing sheet.
- e) Align the sample pad with the bottom edge of the backing sheet and paste it.
- f) The sheet was cut to 5 mm width using a cutting module.
- g) The sheet was put in the housing case.