Supplementary Information

A rapid and highly sensitive fluorescence immunochromatographic test strip for pepsin detection in human hypopharyngeal saliva

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1. Preparation of fluorescence immunochromatographic test strips

1.1 Preparation of the fluorescent microspheres-antibodies conjunctions

In this experiment, carboxylated fluorescent microspheres were activated using EDC/NHS and then conjugated with pepsin detection antibodies. After washing the fluorescent microspheres with 10 mM pH 6.0 MES buffer, 10μL of 1.0 mg/mL EDC and 15 μL of 1.0 mg/mL NHS were added, and activation was carried out at room temperature with shaking for 30 minutes. The mixture was then centrifuged at 4°C and 15,000 rpm for 20 minutes, and resuspended in 0.01 M pH 8.0 borate buffer. 1.0 mg of pepsin detection antibodies were added to the activated fluorescent microspheres (1mL), and the mixture was gently shaken at room temperature for 2 hours. Blockmaster CE510 was added to react for 1.5 hours to block excess sites. After blocking, the fluorescent microspheres-antibodies conjunctions were stored in a microsphere storage solution (50 mM Tris-base + 3% glycine + 1% BSA + 0.1% casein sodium salt + 0.1% Tween 20 + 0.87% NaCl + 0.1% Proclin 300, pH 7.2) for future use.

1.2 Preparation of the coated plate

Capture antibodies were coated on the NC membrane using a spray coating machine. The NC membrane was placed in the center of the backing board, with detection line (T line) and control line (C line) on the NC membrane. The T line was coated with pepsin capture antibodies at a concentration of 1.5 mg/mL, and the C line was coated with goat anti-mouse IgG at 0.5 mg/mL. The coating parameter was set to 1.0 μL/cm. The coated NC membrane was dried in an air drying oven at 45°C for 24 hours and then stored in a moisture-proof cabinet for future use.

1.3 Preparation of the conjugate pad

The fluorescent microspheres-antibodies conjunctions were diluted with a resuspension solution (20 mM phosphate buffer, 1% trehalose, 20% sucrose, 0.87% NaCl, 0.1% EDTA sodium salt, 1% BSA, 0.1% Tween 20, 0.1% Proclin 300, pH 7.2)

and uniformly sprayed onto a glass fiber pad using a spray coating machine. The spraying speed was set to 6.0 μL/cm. The conjugate pad was dried in an air drying oven at 45°C for 16 hours and then stored in a moisture-proof cabinet for future use.

1.4 Assembly of fluorescence immunochromatographic test strips

The fluorescence immunochromatographic test strip was assembled by sequentially sample pad, conjugate pad, NC membrane, and absorbent pad onto a PVC bottom plate. The assembled paste board are then cut into test strips with a width of 3.9 mm using a high-speed continuous cutter, which were then placed in a plastic casing. The assembled test strips were stored in a dry place at room temperature and kept in dark place.