Electronic Supporting Material (ESM)

Sensitive and rapid determination of heat shock proteins 70 using Lateral flow fluorescence immunostrips and upconversion nanoparticles as reporters

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Contents

1. Experimental

1.1 Synthesis of signal probe UCNP-Ab2 conjugate

The signal probe UCNP-Ab2 conjugate was synthesized by covalently coupling the antibodies (Ab2) to the carboxylated UCNP via the condensation reaction between the amino group of the Hsp70 antibody (Ab2) and carboxyl group on the surface of UCNP in three steps.[1,2] Firstly, 1 mL of 10 mg/mL UCNP-PAA aqueous solution was added by a pipette into a disposable test tube (2.5 mL), and then was centrifuged in a high-speed refrigerated centrifuge (1,0000 rmp for 10 min). The supernatant was discarded and then added 1 mL of cleaning solution (3.3 mL dimethyl sulfoxide, 0.2 mL of 1 mM 2-morpholinoethanesulfonic acid and 6.5 mL of ultrapure water), allowed to be ultrasonicated for 2 min for three times. The UCNP-PAA nanoparticles finally re-suspended in 1.0 mL of 1 mM MES (pH= 6.10) buffer. Secondly, 30 μL of 6 mg/mL EDC solution and 30 μL of 6 mg/mL Sulfo-NHS solution were added into 1.0 mL of cleaned UCNP-PAA nanoparticles, and then allowed to stir at constant temperature mixer (400 rmp, 37 °C, 1 h), to activate the surface carboxylation of UCNP-PAA. The activated solution was centrifuged (1,0000 rmp, 10 min), cleaned as described above and then suspended in a 1 mL of 1 mM MES buffer (pH=6.10). Thirdly, 1.0 mL of UCNP-PAA dispersion (10 mg/mL) was mixed with 100 μL of 0.5 mg/mL Hsp70 Ab2 and incubated for 3 h at constant temperature mixer (400 rmp 37 °C). The UCNP-Ab2 conjugates were centrifuged 10 min at 8,000 rmp, and then washed three times with 1×PBS, finally dispersed in 1.0 mL of 1×PBS. The concentration of UCNP-Ab2 conjugate suspension solution was expressed to be 10 mg/mL based on the amount of UCNP-PAA in the solution.

2. Results and discussion

2.1 Feasibility of the method using prepared lateral flow immunostrips

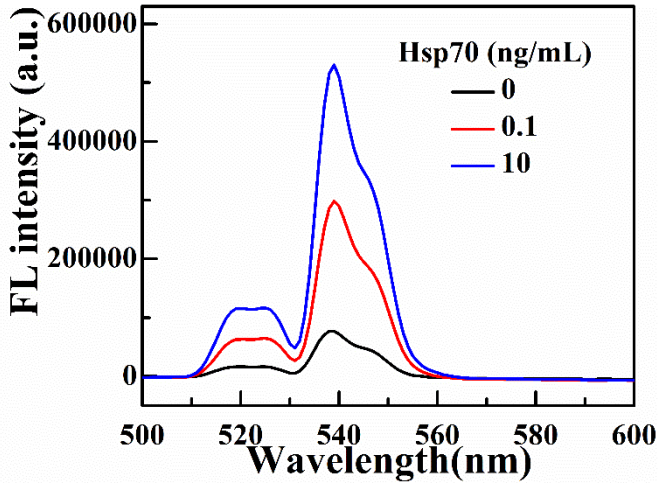

Fig. S1 The representative FL spectra obtained on the test zone. λex=980 nm; λem= 540 nm (10 μL of 10 mg/mL UCNP-Ab2, 2 μL of 2.0 mg/mL Hsp70 Ab1, and 20 μL of Hsp70 solution)

2.2 Reproducibility

Table S1 The obtained PL intensity in the Hsp70 solutions (A-6 nM, B-8 nM) for 7 duplicates

	PL intensity							mean	SD	RSD
A	411697	404594	421211	396944	399222	433386	441984	415576	17304	4.2%
B	524169	501646	533445	553462	541324	469010	537198	526750	21062	4.0%

References

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