

## Electronic Supplementary Information

### Hybridization Chain Reaction based Fluorescence Immunoassay Using DNA

#### Intercalating dye for Signal Readout

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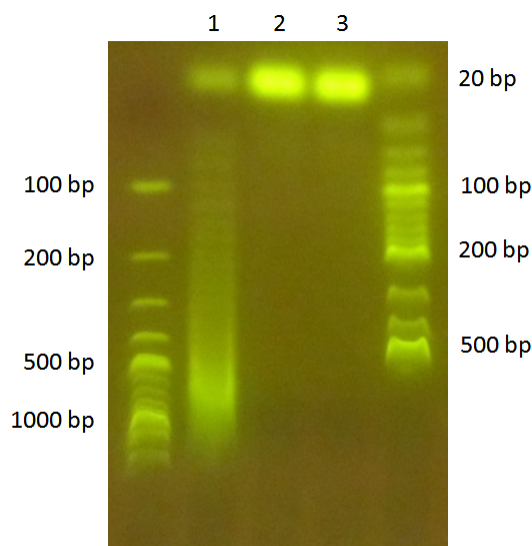


Fig. S1 Native gel electrophoresis of HCR amplified DNA: (1) 0.1  $\mu\text{M}$  Bio-CS, 1  $\mu\text{M}$  H1 and 1  $\mu\text{M}$  H2; (2) 0.1  $\mu\text{M}$  Bio-RS, 1  $\mu\text{M}$  H1 and 1  $\mu\text{M}$  H2; (3) 1  $\mu\text{M}$  H1 and 1  $\mu\text{M}$  H2 in 40  $\mu\text{L}$  reaction buffer.

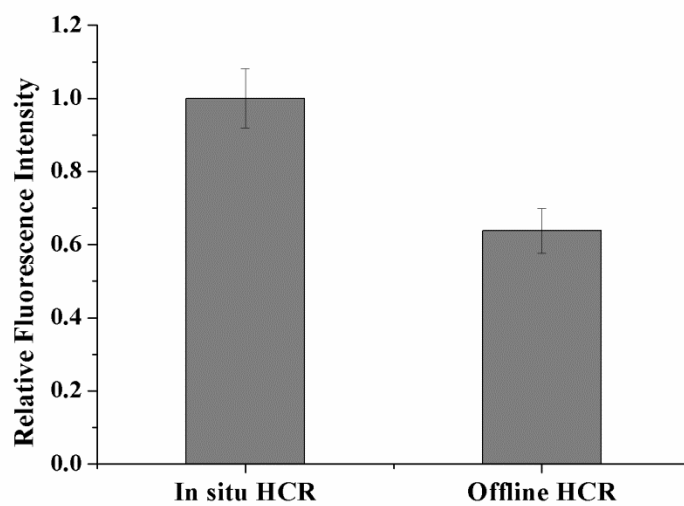


Fig. S2 Comparison between in situ and offline HCR for fluorescence immunoassay in the platform of IgG/Bio-SecAb /Avidin/Bio-CS/H1+H2/GF

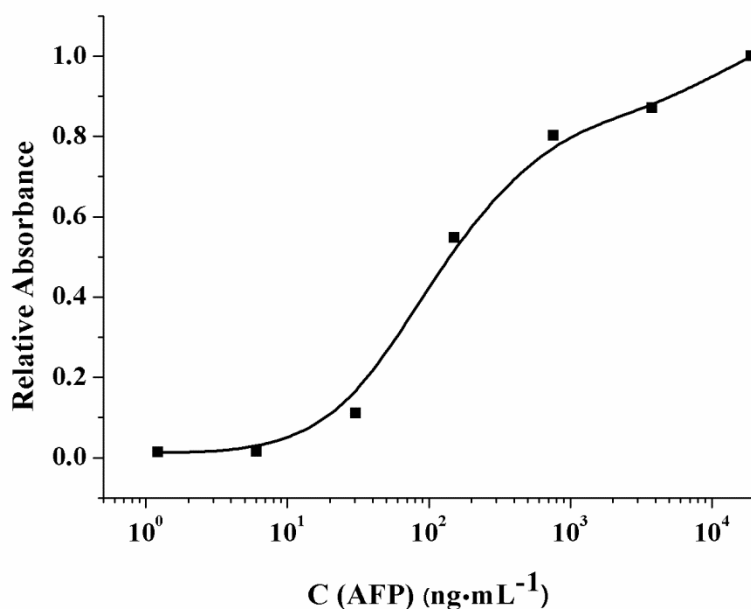


Fig. S3 Traditional ELISA for AFP detection in PBS.

**Table S1 Comparison of different immunoassays for AFP detection**

Technique	Analytical Method	Dynamic Range	LOD
Fluorescence Resonance Energy Transfer <sup>1</sup>	Persistent-luminescence nanoparticles (PLNPS) as photoluminescent probe	0.8~45 ng/mL	0.41 ng/mL
Fluorescence polarization immunoassay <sup>2</sup>	QDs520 labeled AFP	0.5~500 ng/mL	0.28 ng/mL
Near-Infrared Surface-Enhanced Fluorescence (NIR-SEF) <sup>3</sup>	AgNPs/SAv-800CW NIR-SEF	Not given	39.1 pg/mL
Resonance light scattering correlation spectroscopy <sup>4</sup>	Diffusion time changes of antibody labeled AgNPs after immunoreaction	6.9~690 ng/mL	6.9 ng/mL
Electrochemiluminescence (ECL) <sup>5</sup>	CdSe QDs-functionalized ZnO nanoparticles labeled antibody for enhanced ECL signal	0.5~600 ng/mL	0.48 ng/mL
Chemiluminescence <sup>6</sup>	Antibody immobilized carboxylic resin beads coupled with micro-bubble accelerated immunoreaction	1~80 ng/mL	0.1 ng/mL
Chemiluminescence Resonance Energy Transfer <sup>7</sup>	Gold nanoparticle based sandwich immunoassays	5~70 ng/mL	2.5 ng/mL
Fluorescence (This work)	Hybridization chain reaction based fluorescence immunoassay	28 ng/mL-20 µg/mL	6.0 ng/mL

## References

1. B. Y. Wu, H. F. Wang, J. T. Chen and X. P. Yan, *Journal of the American Chemical Society*, 2011, **133**, 686-688.
2. J. N. Tian, L. J. Zhou, Y. C. Zhao, Y. Wang, Y. Peng and S. L. Zhao, *Talanta*, 2012, **92**, 72-77.
3. M. D. Furtaw, D. L. Steffens, T. M. Urlacher and J. P. Anderson, *Analytical Chemistry*, 2013, **85**, 7102-7108.
4. T. Lan, C. Q. Dong, X. Y. Huang and J. C. Ren, *Talanta*, 2013, **116**, 501-507.
5. J. X. Zhang, S. L. Liu, J. C. Bao, W. W. Tu and Z. H. Dai, *Analyst*, 2013, **138**, 5396-5403.
6. X. Pei, B. Chen, L. Li, F. Gao and Z. Jiang, *Analyst*, 2010, **135**, 177-181.
7. X. Y. Huang and J. C. Ren, *Analytica Chimica Acta*, 2011, **686**, 115-120.