Supporting information

High-sensitivity detection of two H7 subtypes of Avian Influenza virus (AIV) by immunochromatographic assay with high chroma red silica nanoparticles

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Materials

Tetraethyl orthosilicate (TEOS, 99.99%), ammonia ($NH_3 \cdot H_2O$, 28.0%), sodium chloride (99.5%), were obtained from Aladdin Industrial Inc. (Shanghai, China). Ethanol was obtained from Xilong Scientific Co., Ltd (Guangdong, China). Ethylene imine polymer (PEI, MW=70000, 30 wt% aqueous solution) and Poly(sodium-p-styrenesulfonate) (PSS, $M_w \approx 70000$, powder) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). The dyes were supplied by Zhejiang Shunlong Chemical Co., Ltd. (Zhejiang, China). Other reagents were all of analytical grade and were used as received without further purification. The water used was deionized water.

Instruments

Hitachi SU8010 scanning electron microscopy (SEM) was purchased from Hitachi Inc. (Tokyo, Japan); Malvern Nano ZS potential laser particle analyzer was provided by Malvern Instruments Co., Ltd. (Worcestershire, UK); UV 2600 UV-vis spectrophotometer was purchased from Shimadzu Co., Ltd (Shanghai, China); Multiskan spectrum was purchased from Thermo Fisher Scientific Inc. (Waltham, USA). A Thermo Nicolet 380 Fourier transform infrared (FTIR) spectrometer was provided by Thermo Fisher Scientific Co.(Shanghai, China).

Synthesis of red SiNPs

Here we used Stöber method for preparing monodisperse SiNPs according to the method reported by Zhu et al. with a slightly modification (Zhu et al.2018). Typically, the mixture of 27.3mL ethanol and 2.7 mL TEOS were quickly poured into the mixture containing 10.3 mL of ethanol, 18 mL of H_2O, and 1.7 mL of ammonia, and reacted for 5 h under a constant temperature magnetic stirrer at 37 °C and 400 rpm. The obtained SiO_2 nanoparticles were centrifuged and washed three times with pure water and ethanol, alternately. After washing, 0.1g SiNPs were ultrasonic dispersed in a mixture of 15 mL H_2O and 80 μL C.I. Direct Red 28 solution (0.1 g/mL), then 3.6~ 3.75 mL polyelectrolyte (PEI, 1 g/L, 0.1 mol/L NaCl) was added. The reaction mixture was kept to stand at room temperature for 25 min. The particles were then centrifuged (3min, 8000 rpm) and washed

several times with water to obtain monolayer red SiNPs and dilute to 2 mL of pure water for later use.

Synthesis of high chroma red SiNPs

Scheme. 1SA shows the brief process of preparing the high chroma red SiNPs. In brief, the red SiNPs prepared above were dissolved in 15 mL of H_2O, added 100 μL of PEI under ultrasonic conditions. After deposited at room temperature for 10 min, centrifuged and washed. Then redispersed in 15 mL H_2O, added 3.6~3.75 mL Poly (sodium-p-styrenesulfonate) (PSS, 1 g/L, 0.2 mol/L NaCl) under ultrasonic conditions, deposited for 25 min, the mixture was isolated by centrifugation and washed several times with water. At this time, a negatively charged polyelectrolyte film was coated on the surface of the red SiNPs. This whole process describes the assembly of monolayer dyes coated by bimolecular nano-films: $(PEI/PSS)_1$ red SiNPs and repeated three more times to obtain high chromaticity red SiNPs coated with different number of nano-bilayer films.

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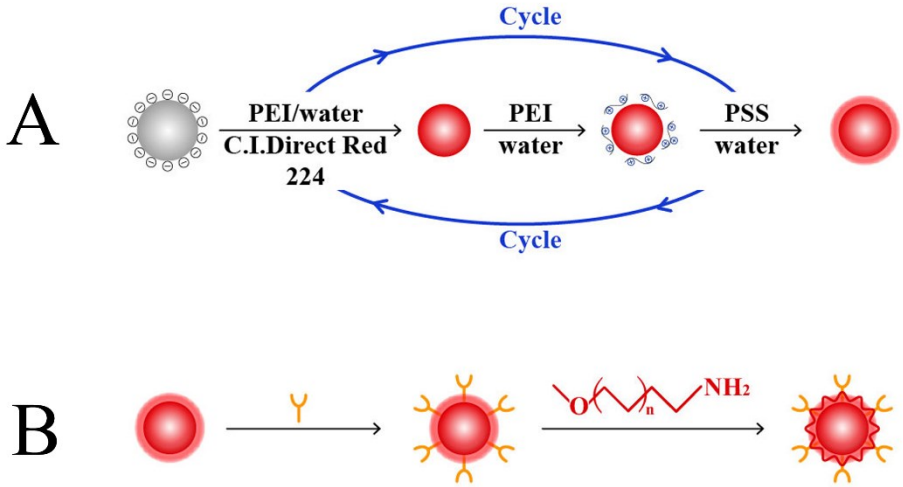

Scheme. 1 A schematic illustration of the fabrication process of high chroma red SiNPs **(A)**, The formation of mAb1-modified high chromatic red SiNPs probes **(B)**,

55 [1] Z. Su, G. Zhao and W. Dou, *Mikrochim Acta*, 2020, **187**, 658.

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CERTIFICATE OF ANALYSIS

名　　称	H7 单抗-标记	货　　号	BND15-1
描　　述	抗甲型流感病毒（Flu A）H7 亚型血凝素（HA）蛋白		
免 疫 原	Flu A-H7-HA 天然抗原		
来　　源	小鼠腹水	克　　隆	单克隆
缓 冲 液	10mM PBS（7.4）	抗体类型	IgG1
纯化方式	蛋白 A 亲和纯化	批　　号	20171220
生产日期	2017-12-20	有效期至	2020-12-20
特 异 性	仅识别甲型流感病毒 H7 亚型（H7N2、H7N9），与甲型流感病毒的其它亚型（H1、H3、H5、H9）、乙型流感病毒以及其它病原体无交叉反应。		
敏 感 性	与推荐的抗体配对使用，最低检出限可达： 1ng/ml（H7-HA 抗原）或 1.0×10^3PFU/ml（H7 病毒培养液）		
应　　用	ELISA、免疫层析，其它方法未验证。		
储存条件	短期储存（1 周），2-8℃；长期储存需-20℃冻存，避免反复冻融。		

质量控制

项目	方法	结果
外观	目测	澄清液体
分子量	SDS-PAGE	~150KD
纯度	SDS-PAGE	95%
浓度	C=A280×0.75×稀释比例	6.0mg/ml
效价	间接 ELISA	1:600 000
结论	符合要求	

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CERTIFICATE OF ANALYSIS

名　　称	H7 单抗-包被	货　　号	BND15-2
描　　述	抗甲型流感病毒（Flu A）H7 亚型血凝素（HA）蛋白		
免 疫 原	Flu A-H7-HA 天然抗原		
来　　源	小鼠腹水	克　　隆	单克隆
缓 冲 液	10mM PBS（7.4）	抗体类型	IgG1
纯化方式	蛋白 A 亲和纯化	批　　号	20171118
生产日期	2017-11-18	有效期至	2020-11-18
特 异 性	仅识别甲型流感病毒 H7 亚型（H7N2、H7N9），与甲型流感病毒的其它亚型（H1、H3、H5、H9）、乙型流感病毒以及其它病原体无交叉反应。		
敏 感 性	与推荐的抗体配对使用，最低检出限可达： 1ng/ml（H7-HA 抗原）或 1.0×10^3PFU/ml（H7 病毒培养液）		
应　　用	ELISA、免疫层析，其它方法未验证。		
储存条件	短期储存（1 周），2-8℃；长期储存需-20℃冻存，避免反复冻融。		

质量控制

项目	方法	结果
外观	目测	澄清液体
分子量	SDS-PAGE	~150KD
纯度	SDS-PAGE	95%
浓度	C=A280×0.75×稀释比例	5.5mg/ml
效价	间接 ELISA	1:550 000
结论	符合要求	

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