

Theo yêu cầu của khách hàng, trong một năm qua, chúng tôi đã dịch qua 16 môn học, 34 cuốn sách, 43 bài báo, 5 sổ tay (chưa tính các tài liệu từ năm 2010 trở về trước) Xem ở đây

**DỊCH VỤ
DỊCH
TIẾNG
ANH
CHUYÊN
NGÀNH
NHANH
NHẤT VÀ
CHÍNH
XÁC
NHẤT**

Chỉ sau một lần liên lạc, việc dịch được tiến hành

Giá cả: có thể giảm đến 10 nghìn/1 trang

Chất lượng: Tao dựng niềm tin cho khách hàng bằng công nghệ 1. Bạn thấy được toàn bộ bản dịch; 2. Bạn đánh giá chất lượng. 3. Bạn quyết định thanh toán.

Tài liệu này được dịch sang tiếng việt bởi:

www.mientayvn.com

Từ bản gốc:

<https://drive.google.com/folderview?id=0B4rAPqlxIMRDfIBVOnk2SHNlbkR6NHJiN1Z3N2VBaFJpbnlmbjhgQ3RSc011bnRwbUxsczA&usp=sharing>

Liên hệ dịch tài liệu :

thanhlam1910_2006@yahoo.com hoặc frbwrthes@gmail.com hoặc số 0168 8557 403 (gặp Lâm)

Tìm hiểu về dịch vụ: http://www.mientayvn.com/dich_tiang_anh_chuyen_nghanh.html

Rapid and Sensitive Detection of Protein Biomarker Using a Portable Fluorescence Biosensor Based on Quantum Dots and a Lateral Flow Test Strip

Phát hiện nhanh và nhạy Biomarker Protein bằng cảm biến sinh học huỳnh quang cầm tay cấu thành từ các chấm lượng tử và que thử nhanh Biomarker: dấu ấn sinh học protein biomarker là số phân tử sinh học chỉ rõ tình trạng, diễn tiến của

MATERIALS AND METHODS

Reagents and Materials.

Human ceruloplasmin was purchased from Genway Biotech, Inc. (San Diego, CA). Goat antinitrotyrosine antibody was obtained from Cayman Chemical Company (Ann Arbor, MI) while polyclonal human ceruloplasmin antibody was ordered from Abcam Inc. (Cambridge, MA). A nitrocellulose membrane, absorbent pad, sample pad, conjugation pad, and backing cards were purchased from Millipore (Bendford, MA). Casein (1%) was provided by Bio-Rad (Hercules, CA). A Qdot 585 antibody conjugation kit was obtained from Invitrogen. The reagent components in the kit include Qdot 585 nanocrystals, dithiothreitol (DTT) stock solution, succinimidyl trans-4-(N-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC) stock solution, 2-mercaptoethanol, dye-labeled marker for antibody elution, separation media, and exchange buffer. All other chemicals were ordered from Sigma-Aldrich without further purification. All buffers and reagent solutions were prepared with purified water, which was produced from Barnstead Nanopure System (Kirkland, WA).

Instruments.

LM5000 Laminator, XYZ-3050 dispenser consisting of AirJet Quanti 3000 and BioJet Quanti 3000, and Guillotine Cutting System CM 4000 were from

bệnh

VẬT LIỆU VÀ PHƯƠNG PHÁP

Hóa chất và vật liệu.

Ceruloplasmin người mua từ Genway Biotech, Inc. (San Diego, CA). Kháng thể antinitrotyrosine dê mua từ Công Ty Hóa Chất Cayman (Ann Arbor, MI) còn kháng thể ceruloplasmin người đa dòng mua từ Abcam Inc. (Cambridge, MA). Màng nitrocellulose, màng hấp thụ, màng hút mẫu, màng chứa chất cộng hợp, và các tấm đỡ mua từ Millipore (Bendford, MA). Casein (1%) do Bio-Rad (Hercules, CA) cung cấp. Bộ liên hợp kháng thể Qdot 585 mua từ Invitrogen. Thành phần hóa chất trong bộ bao gồm các tinh thể nano Qdot 585, dung dịch gốc dithiothreitol (DTT), dung dịch gốc succinimidyl trans-4-(N-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC), 2-



Biodot Ltd. (Irvine, CA). A portable fluorescence strip reader ESE-Quant FLUO was purchased from DCN Inc. (Irvine, CA). A bench-top Eppendorf 5804 centrifuge was obtained from Eppendorf (Hauppauge, NY). An Ultrospec 2100 Pro UV/Visible spectrophotometer was provided by Blochrom Ltd. (Cambridge, England).

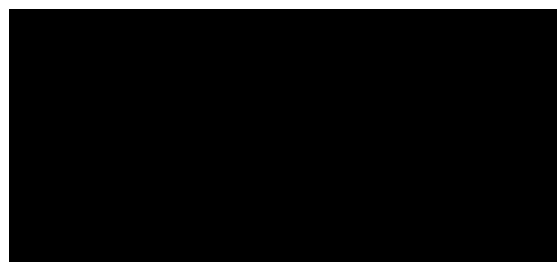
Preparation of Qdot-Antinitrotyrosine Conjugate.

Qdot-antinitrotyrosine conjugate was prepared according to the protocol for the Qdot antibody conjugation kit from Invitrogen. Briefly, 125 yL of Qdot 585 nanocrystals were first activated with 14 yL of 10 mM SMCC at room temperature for 1 h, followed by being desalted with a NAP-5 desalting column in the presence of exchange buffer as elution solvent. The colored eluate (~ 500 yL) was then collected into a centrifuge tube. At room temperature, 300 yL of 1 mg/mL goat antinitrotyrosine antibody was reduced with 20 mM DTT for 0.5 h. The resulted mixture was incubated with a dye-labeled marker and purified with a NAP-5 desalting column. The colored fraction (~500 yL) was collected and then mixed with the activated Qdot nanocrystals at room temperature for 1 h to form the conjugation complex, followed by the addition of 10 yL of 10 mM 2-mercaptoethanol to quench the reaction. The quenched conjugation mixture was then split into two ultrafiltration devices and concentrated to ~20 yL for each

half mixture by centrifuging at 7000 rpm on a benchtop Eppendorf centrifuge. Following the instructions from the conjugation kit, separation media was gently loaded into the column and then conditioned with water and PBS, respectively. The concentrated conjugation mixture was then pipetted into the size-Position on Test Strip

Figure 1. (A) Schematic illustration of the test strip and (B1-B4) the detection of nitrated ceruloplasmin using fluorescent Qdot-based FLFTS. (B1) Aqueous sample containing nitrated ceruloplasmin is applied to sample pad. (B2) Nitrated ceruloplasmin combines with QD-antinitrotyrosine conjugate and also migrates along the porous membrane by capillary action. (B3) Nitrated ceruloplasmin is captured by anticerculoplasmin antibodies immobilized on the test line. The excess Qdot conjugates continue to migrate toward the absorption pad. (B4) Fluorescence signal of Qdot is detected using a test strip reader (solid line). As a control, ceruloplasmin without nitration cannot be recognized by Qdot-antinitrotyrosine conjugates, so no fluorescence signal can be seen on the test strip (dotted line).

exclusion column followed by the addition of PBS. During the elution by gravity, the first ten drops only of colored conjugate (~200 μ L) was collected into a centrifuge tube and stored at 4 °C until use.



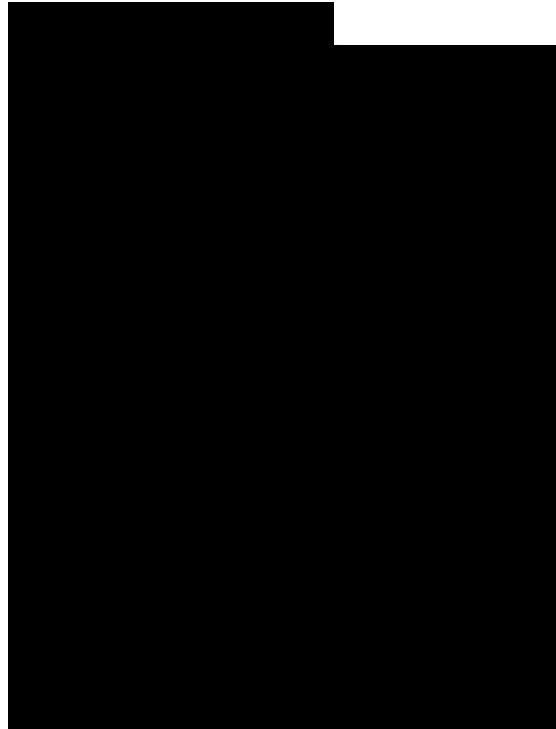
Following the instructions from the protocol for the Qdot antibody conjugation kit from Invitrogen, the conjugate concentration was determined by measuring the absorbance density of the conjugate at 585 nm with a Ultrospec 2100 Pro UV/Visible spectrophotometer and, then, using the formula $A = \epsilon cL$, where A is the absorbance, ϵ is the molar extinction coefficient, c is the molar concentration of Qdot conjugate, and L is the path length of the cuvette.

Nitration of Ceruloplasmin.

One mg/mL Human ceruloplasmin in phosphate buffered saline (pH 7.4) was nitrated by bolus addition of 1 mM authentic peroxyntirite (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations. The volume of added peroxyntirite was <1% of the total volume of the incubation mixture, and the reaction was quenched by the addition of 0.7 M mannitol.

Fabrication of Qdot-Based FLFTS.

Qdot-based FLFTS is composed of sample application pad, conjugation pad, nitrocellulose membrane, absorption pad, and a backing card as shown in Figure 1A. Both the sample pad (20 mm x 30 cm) and conjugation pad (8 mm x 30 cm) were made from glass fiber. The conjugation pad was prepared by dispensing a desired volume of Qdot-antinitrotyrosine onto the glass fiber pad using the BioJet Quanti 3000 dispenser, followed by being dried at room temperature and then stored at 4 °C. Meanwhile, 1 mg/mL



polyclonal human ceruloplasmin antibody was dispensed onto the test line of the nitrocellulose membrane (40 mm x30 cm) using a BioJet Quanti 3000 dispenser. After 1 h drying at 4 °C, the membrane was blocked with 1% casein at room temperature for 30 min and then dried under vacuum for 40 min. Then, all of the parts were assembled on a plastic adhesive backing card using the Batch Laminating System LM5000, and each part overlapped 2 mm to ensure the solution migrating through the entire strip during the assay. Finally, the Qdot-based FLFTS with a 4 mm width was cut using Guillotine Cutting System CM 4000 and assembled into a strip cassette for the following assay.

Fluorescence Assay Procedure of Nitrated Ceruloplasmin. Fifty yL of sample solution containing a desired concentration of nitrated ceruloplasmin in running buffer (1x PBS with 6% BSA) was added onto the sample pad. Ceruloplasmin without nitration was used here as control. Both the sample and control solution were migrated toward the absorption pad by capillary action. After about 10 min, the cassette containing the test strip was inserted into an ESE-Quant FLUO reader, followed by the recording of fluorescence intensity from Qdot on the test line to quantify the analytes. For the detection of nitrated ceruloplasmin in human plasma, 20 times diluted plasma spiked with different quantities of nitrated ceruloplasmin

was added onto the sample pad. The results were obtained by reading the optical response with the strip reader after 10 min. Meanwhile, these strips after assay were put under a UV light, and the corresponding fluorescence images were captured directly by a Sony DSLR-A300 digital camera.

RESULTS AND DISCUSSION

Assay Principle of Qdot-Based FLFTS. Figure 1 schematically illustrates the configuration and measuring principle of Qdot-based FLFTS. This FLFTS is composed of sample application pad, conjugation pad, absorption pad, and nitrocellulose membrane (Figure 1A). All the components were assembled onto a plastic adhesive backing card. During the assay, aqueous sample containing nitrated ceruloplasmin was applied onto the sample application pad as shown in Figure 1B1. Subsequently, the analyte migrated along the porous membrane by capillary action and then bound with Qdot-antinitrotyrosine on the conjugation zone according to the specific antibody-antigen interaction (Figure 1B2). The formed complexes continued to migrate along the membrane and were captured by anticерuloplasmin antibodies, which resulted in the accumulation of Qdot on the test line (Figure 1B3). The excess Qdot conjugates continue to flow into the absorption pad to the end of the strip. Quantitative analysis was realized by reading the fluorescence intensities of test line with a portable strip reader (Figure



1B4). The more analyte in the sample, the more Qdot conjugates would be captured to the test line, which leads to the increase of fluorescence intensity. According to the principle described above, the fluorescence intensity on the test line would be proportional to the concentration of nitrated ceruloplasmin in the samples.

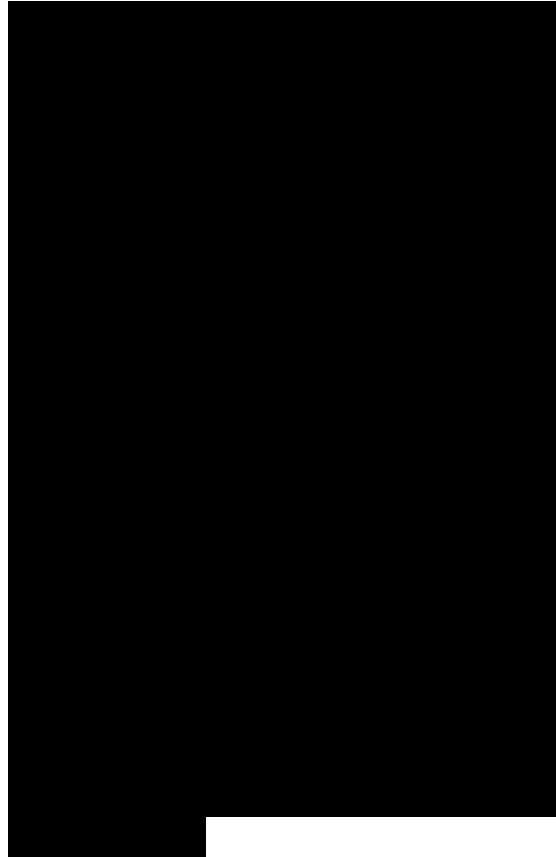
Figure 2 shows the typical corresponding responses of Qdot-based FLFTS to different concentrations of nitrated ceruloplasmin. Here, human ceruloplasmin without nitration served as a control. As shown in this figure, well-defined curves were observed in the presence of nitrated ceruloplasmin and the peak area was getting larger as the target concentration increased from 10 to 100 ng/ mL because more Qdot was captured on the test line based on the mechanism of Qdot-based FLFTS. In contrast, the presence of ceruloplasmin without nitration did not contribute to the signal and exhibited a very low background. The results indicate the great possibility of Qdot-based FLFTS for sensitive protein detections.

Determination of Qdot Conjugate Concentration.

Following the instructions for the Qdot antibody conjugation kit from Invitrogen, the Qdot conjugate eluting from the final column could be determined according to the Beer-Lambert law by measuring the absorbance density of the conjugate at 585 nm:

$$A = ecL$$

Where A is the absorbance, e is the



molar extinction coefficient (as 400 000 M⁻¹ cm⁻¹ provided by Invitrogen for Qdot 585), c is the molar concentration, and L is the path length of the cuvette. The result shows that the Qdot conjugate has $A = 0.6$ measured in a cuvette with 1 cm path length, so $c = A/e = 0.6/400\ 000 = 1.5 \times 10^{-6}$ M, which is the original concentration of as-prepared Qdot conjugate stock solution.

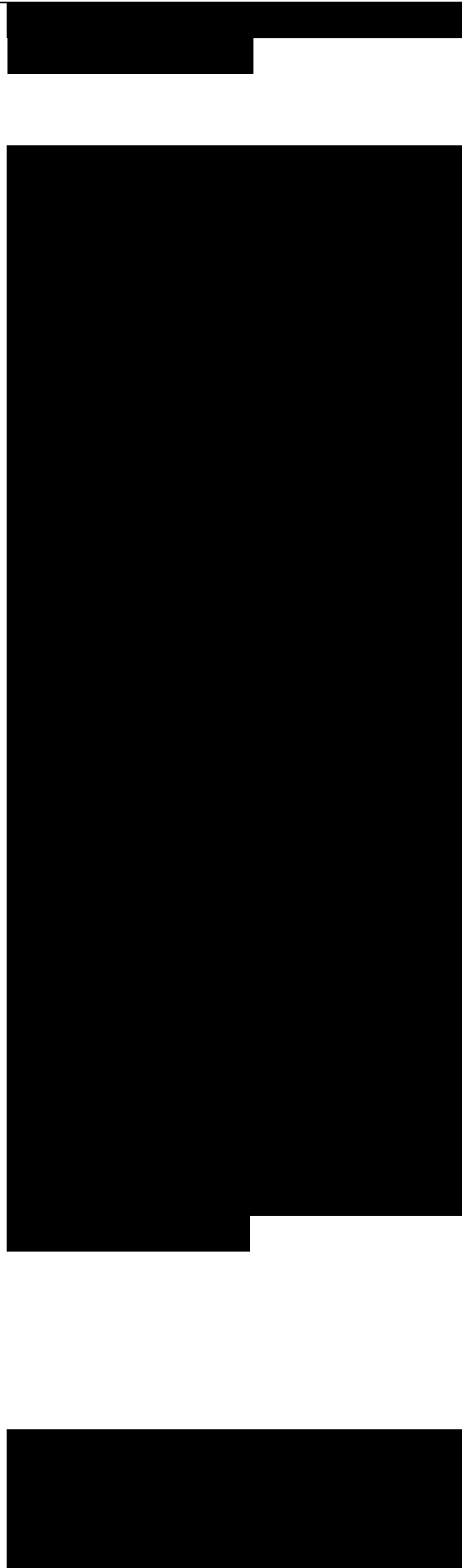
Parameter Optimization. The amount of Qdot-antinitrotyrosine, loaded on the glass fiber by physical absorption, directly affects the fluorescence response of Qdot-based FLFTS since the signal mainly depends on the amount of Qdot-antinitrotyrosine conjugates captured on the test line. To probe the optimal amount of Qdot conjugates for the assay, we diluted them into various concentrations and investigated the influence on signal-to-noise (S/N) ratio of the biosensor for 1 μ Mg/mL nitrated human ceruloplasmin. Ten μ Mg/mL non-nitrated ceruloplasmin served here as a control. As can be seen from Figure 3, the S/N ratio was found to be highest for dispensing 150 nM Qdot-antinitrotyrosine. However, the decrease of S/N at a higher concentration is resulted from the increase of background signal due to too high of a concentration of Qdot-antinitrotyrosine conjugate while that at

lower concentration is ascribed to the decrease of signal due to too low of an amount of Qdot conjugate availability. Therefore,

150 nM Qdot-antinitrotyrosine conjugate was routinely used as the optimal concentration throughout the entire study.

Nonspecific binding is one of the likely hindrances in the development of nanoparticle-based immunoassays. In the current experiment, there was a fluorescence response obtained from the control sample (ceruloplasmin without nitration), which resulted from the nonspecific binding of Qdot conjugates on the test line. To obtain a maximum response and a minimum nonspecific absorption, we found that blocking the nitrocellulose membrane with 1% casein could eliminate the influence of nonspecific binding. Figure 4 displays the corresponding response of Qdot-based FLFTS for 10 yg/mL ceruloplasmin with (A) and without (B) nitration before (dashed line) and after (solid line) blocking. The results show that the nonspecific binding was effectively reduced after the blocking while bright fluorescence was well maintained for the target sample. The significant removal of nonspecific adsorption maybe attributed to the shield effect of casein, which was successfully absorbed onto the surface of membrane pad. As a result, the blocking with 1% casein was employed for the following experiments.

It is important to evaluate the effect of polyclonal human ceruloplasmin antibody concentration on the performance

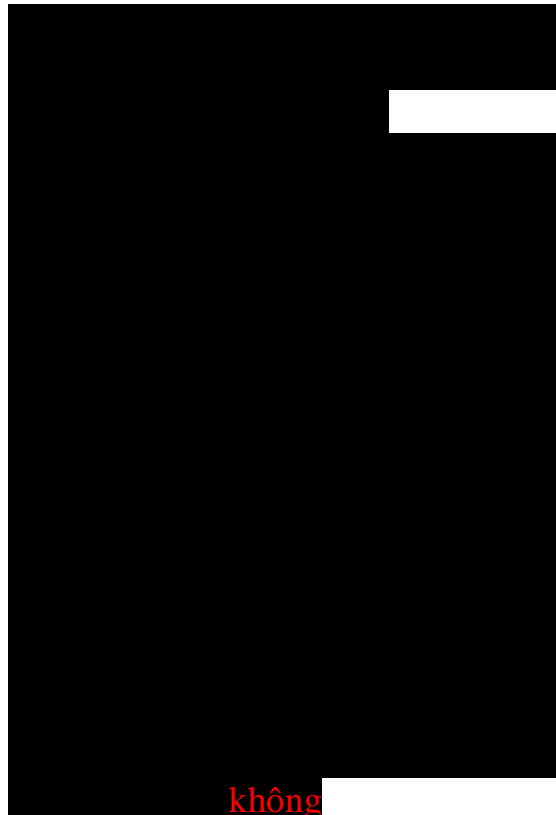


of this biosensor. Consequently, we have investigated different concentrations of ceruloplasmin antibody (10 ng/mL, 100 ng/mL, 500 ng/mL, 1 mg/mL, 2 mg/mL, and 3 mg/mL) and found that 1 mg/mL has the best response for the detection of nitrated ceruloplasmin. Furthermore, we have studied the stability of this biosensor by periodical testing and noticed that this test strip could be stored at 4 °C (sealed) for at least 3 months and still maintain good performance.

Analytical Performance of Qdot-Based FLFTS for Nitrated Ceruloplasmin Detection.

To investigate the ability of Qdot-based FLFTS for protein sensitive quantification, the assay was examined with different concentrations of nitrated ceruloplasmin. The fluorescence intensity on test line was recorded and plotted as a function of various concentrations of nitrated ceruloplasmin. Figure 5 shows the typical response of this biosensor within 10 min for nitrated ceruloplasmin with different concentrations of 1 ng/mL, 5 ng/mL, 10 ng/mL, 40 ng/mL, 100 ng/mL, 1 yg/mL, and 10 yg/mL, respectively. Ten yg/mL ceruloplasmin without

Figure 5. Qdot-based FLFTS response for 10yg/mL, 1 yg/mL, 100 ng/mL, 40 ng/mL, 10 ng/mL, 5 ng/mL, and 1 ng/mL nitrated cerulo-plasmin (curves a-g) and 10 yg/mL ceruloplasmin without nitration (curve h), respectively.



nitration was used as a control. As can be seen from the figure, well-defined peaks were observed and the peak area increased along with the increasing of target concentration while no obvious fluorescence signal for the control sample could be detected. Meanwhile, trace amount of nitrated ceruloplasmin as low as 1 ng/ mL could be responded by this portable biosensor with a 10 min assay time.

Due to the superior signal brightness and high photostability of Qdot, fluorescence imaging of this biosensor after assay could be employed as a conventional approach to qualify or semiquantify protein analytes visually and rapidly. By observing the fluorescence image directly, we can easily judge the existence or not of target proteins. As shown in Figure 6, the fluorescence band occurred clearly in the presence of nitrated ceruloplasmin. We observed proportional changes in fluorescence brightness of the test lines associated with the concentration of nitrated ceruloplasmin. Such an observation is expected because the test line captures more Qdot conjugates when the analyte concentration is higher. Furthermore, a red signal band from the target concentration as low as 10 ng/mL could be easily seen even with visual inspection. In the presence of ceruloplasmin without nitration, no obvious fluorescence band appeared, indicating a very low nonspecific



cảm biến sinh học
này có hấp thụ không đặc hiệu
thấp

absorption of this biosensor. Accordingly, well-defined peaks were recorded by the strip reader shown on the bottom of Figure 6. Consequently, the strip reader and fluorescence imaging can be combined together to show the assay results of Qdot-based FLFTS with high sensitivity and specificity. Either of them could also be used alone depending on the conditions and requirements. By employing the dual reading approaches, the proposed sensing platform will be a universal and simple strategy for complicated protein analysis.

Determination of Nitrated Ceruloplasmin in Human Plasma. To explore the feasibility of Qdot-based FLFTS for clinical application, the device was then applied to detect nitrated ceruloplasmin spiked in 20-fold diluted human plasma with different concentrations such as 10 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$, 100 ng/mL , 10 ng/mL , and 1 ng/mL , respectively. Ten $\mu\text{g/mL}$ non-nitrated ceruloplasmin served as control. These samples were applied to Qdot-based FLFTS, and the fluorescence signals were recorded by test strip reader and digital camera after 10 min. A good calibration curve was obtained in a wide range as showed in Figure 7. The detection limit was 0.4 ng/mL ($S/N = 3$), which is calculated as the concentration corresponding to 3 times the SD (standard deviation) of the background signal. Error bars are based on six duplicated measurements of nitrated

ceruloplasmin at different concentrations, and the control was also run six replicates. Considering 20-fold dilution of plasma sample during the assay, the detection limit of 0.4 ng/mL is equivalent to 8 ng/mL for undiluted plasma, which is comparable to the value indicated by other techniques for nitrated protein detection such as nitrated fibrinogen and BSA.^{43,44}

Regarding the detection limit of this biosensor and the high concentration (0.5%, i.e., 2.27 μ M) of ceruloplasmin in human plasma, the sensitivity of this assay is sufficient to detect as low as 0.03% nitration of ceruloplasmin in human plasma samples even if only one nitration site is present in the protein. Meanwhile, fluorescence images were also easily observed as shown in Figure 8, where the fluorescence band occurred clearly in the presence of nitrated ceruloplasmin and almost no fluorescence band came up for non-nitrated ceruloplasmin. Furthermore, the fluorescence band on the test line for 10 ng/mL nitrated ceruloplasmin in human plasma could be directly

ABC

Figure 8. Fluorescence imaging of Qdot-based FLFTS for (A) 100 ng/mL and (B) 10 ng/mL nitrated ceruloplasmin and (C) 10 μ g/mL ceruloplasmin without nitration in human plasma.

viewed by naked eyes, which is equivalent to 200 ng/mL for an undiluted plasma sample. Successfully detecting the spiked

human plasma samples using a strip reader or fluorescence imaging displays the promise of Qdot-based FLFTS for various clinical applications in the near future.

CONCLUSIONS

In summary, Qdot as a promising alternative reporter was successfully integrated with lateral flow tests strip and first developed for rapid, sensitive, and one-step quantitative detection of a trace amount of nitrated ceruloplasmin. This portable biosensor takes advantage of the speed and low cost of conventional immunochromatographic strip as well as high sensitivity and photostability of Qdot-based fluorescent immunoassay. Under optimal conditions, this proposed Qdot-based FLFTS is capable of detecting a minimum of 1 ng/mL nitrated ceruloplasmina within 10 min. Furthermore, the linear relationship between peak area and the logarithm of target concentration was observed in the range of 1 ng/mL to 10 yg/mL with a detection limit of 0.4 ng/ mL in a spiked plasma sample, which is equivalent to 8 ng/mL for undiluted plasma. Moreover, the presence of non-nitrated ceruoloplasmin showed no effect on the biosensor response, illustrating the good selectivity. Overall, the Qdot-based FIFTS, considered as an advance in alternative immunosensors, has a great potential for rapid, sensitive, and portable analysis of other protein biomarkers in clinical diagnostics, basic discovery, and a

variety of other biomedical applications.	
---	--