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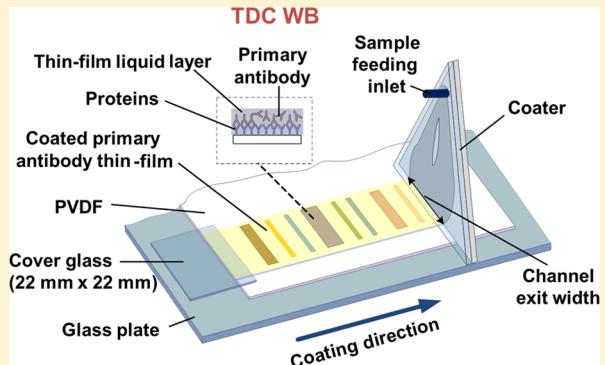
## Western Blotting by Thin-Film Direct Coating

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### Supporting Information

**ABSTRACT:** A novel thin-film direct coating (TDC) technique was developed to markedly reduce the amount of antibody required for Western blotting (WB). Automatic application of the technique for a few seconds easily and homogeneously coats the specific primary antibody on the polyvinylidene fluoride (PVDF) membrane. While conventional WB requires 0.4  $\mu\text{g}$  of the primary antibody, the proposed technique only uses  $4 \times 10^{-2} \mu\text{g}$ , which can be reduced further to  $4 \times 10^{-5} \mu\text{g}$  by reducing the coater width. Moreover, the proposed process reduces antibody probing times from 60 to 10 min. The quantification capability of TDC WB showed high linearity within a 4-log<sub>2</sub> dynamic range for detecting target antigen glutathione-S-transferase. Furthermore, TDC WB can specifically detect the extrinsic glutathione-S-transferase added in the *Escherichia coli* or 293T cell lysate with better staining sensitivity than conventional WB. TDC WB can also clearly probe the intrinsic  $\beta$ -actin,  $\alpha$ -tubulin, and glyceraldehyde 3-phosphate dehydrogenase, which are usually used as control proteins in biological experiments. This novel technique has been shown to not only have valuable potential for increasing WB efficiency but also for providing significant material savings for future biomedical applications.



Western blotting (WB) is a powerful analytical tool that has been widely employed for decades in both scientific research and medical diagnosis. In life science research, it is often used to detect and determine target proteins in cells or tissues.<sup>1–4</sup> WB has also been applied to medical diagnostics, such as blood testing for HIV<sup>5</sup> or Lyme disease in blood samples.<sup>6</sup> WB has several advantages such as high sensitivity and reliability<sup>7</sup> but requires large amounts of target antigens and specific antibodies for testing.

Several automated methods have been reported to reduce the amount of reagents needed for conducting WB.<sup>8–13</sup> A semiautomatic protein detection system was demonstrated using a vacuum to actively drive the blocking reagent and antibody solution through the membrane. This method can complete WB in 30 min but consumes more energy without reducing the required amount of primary antibody.<sup>9</sup> Another method used capillary isoelectric focusing to separate protein samples, which were subsequently immobilized by photo-activated cross-linking to the capillary surface for detection by flowing specific antibodies and reagents through the capillary.<sup>10</sup> In addition to improved sensitivity and automation, this method also provided better experimental reproducibility, but the system is quite expensive to implement and does not provide the size-based separation available in conventional WB. Recently, a method named capillary electrophoresis-Western blot (CEWB) has been shown to efficiently separate samples based on their size-to-charge ratios in a small capillary. CEWB eliminates the electro-blotting step, but the approach needs

further improvements in terms of robustness, throughput, and detection limit. Moreover, CEWB implementation in biochemical laboratories is limited by its complicated apparatus and procedures.

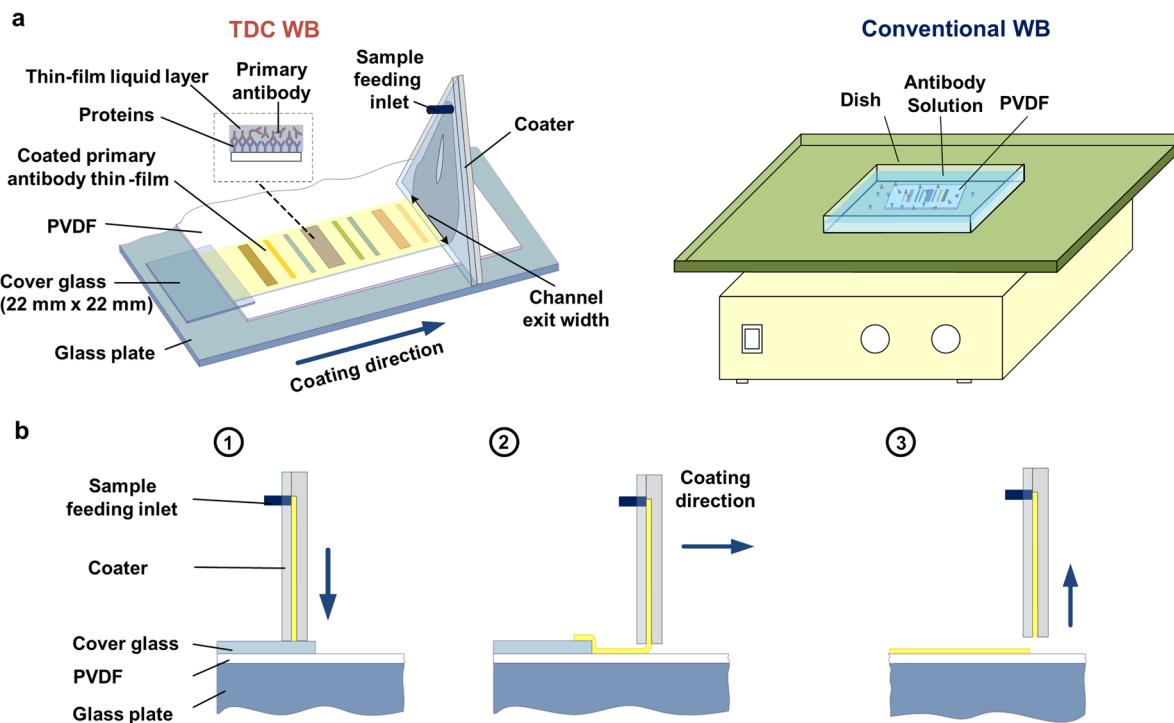
Recently, microfluidic technologies have also been applied to WB.<sup>5,11–13</sup> He et al.<sup>11,12</sup> integrated microfluidic devices for separation, transfer, and blotting operations. This method used immobilized antibodies (about 1  $\mu\text{g}$ ) in a chip to identify the target protein and can detect free prostate specific antigen (500 nM) within 5 min. However, this approach only detects one protein from a single sample and does not provide the protein's molecular size. Hughes and Herr<sup>5</sup> further developed an advanced  $\mu$ Western blotting assay in a single glass microfluidic platform with full automatic controls to analyze complex protein samples from human sera and crude cell lysate. This approach offers advantages of short operational durations (10–60 min), high sensitivity at the femtogram level, and wide quantitation capability over a 3.6-log dynamic range with a lower detection limit of 50 pM sample concentration. Recently, Pan et al.<sup>13</sup> integrated conventional protein blotting and microfluidic immunoassay techniques to analyze the expression of multiple proteins in one sample and demonstrated a significant reduction of the needed antibody amount. However,

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**Figure 1.** Schematic diagram of TDC Western blot (WB): (a) comparison of probing stages for conventional WB and TDC WB and (b) coating processes of TDC WB.

application to general laboratories is limited by requirements for special micromachining and device-interfacing techniques.

Thin film wet coating techniques have been widely used for the mass production of uniformly functional outer layers for various industrial products.<sup>14</sup> In a recent important breakthrough, a new light and thin coater (about 2.3 g and about 1 mm thick for 2 cm-wide coater) has been shown to be useful, material-effective, and user-friendly for laboratory use.<sup>15,16</sup> This new coating technology was applied in this work to develop a thin-film direct coating (TDC) WB that combines thin film immunoassay with conventional protein blotting methods. We demonstrate that the newly developed TDC coater can easily and homogeneously coat the antibody solution on a polyvinylidene fluoride (PVDF) membrane, thus significantly reducing antibody consumption. In addition, the proposed approach is user-friendly, is easy to operate, and provides improved immunoassay results in just seconds.

## EXPERIMENTAL DESCRIPTIONS

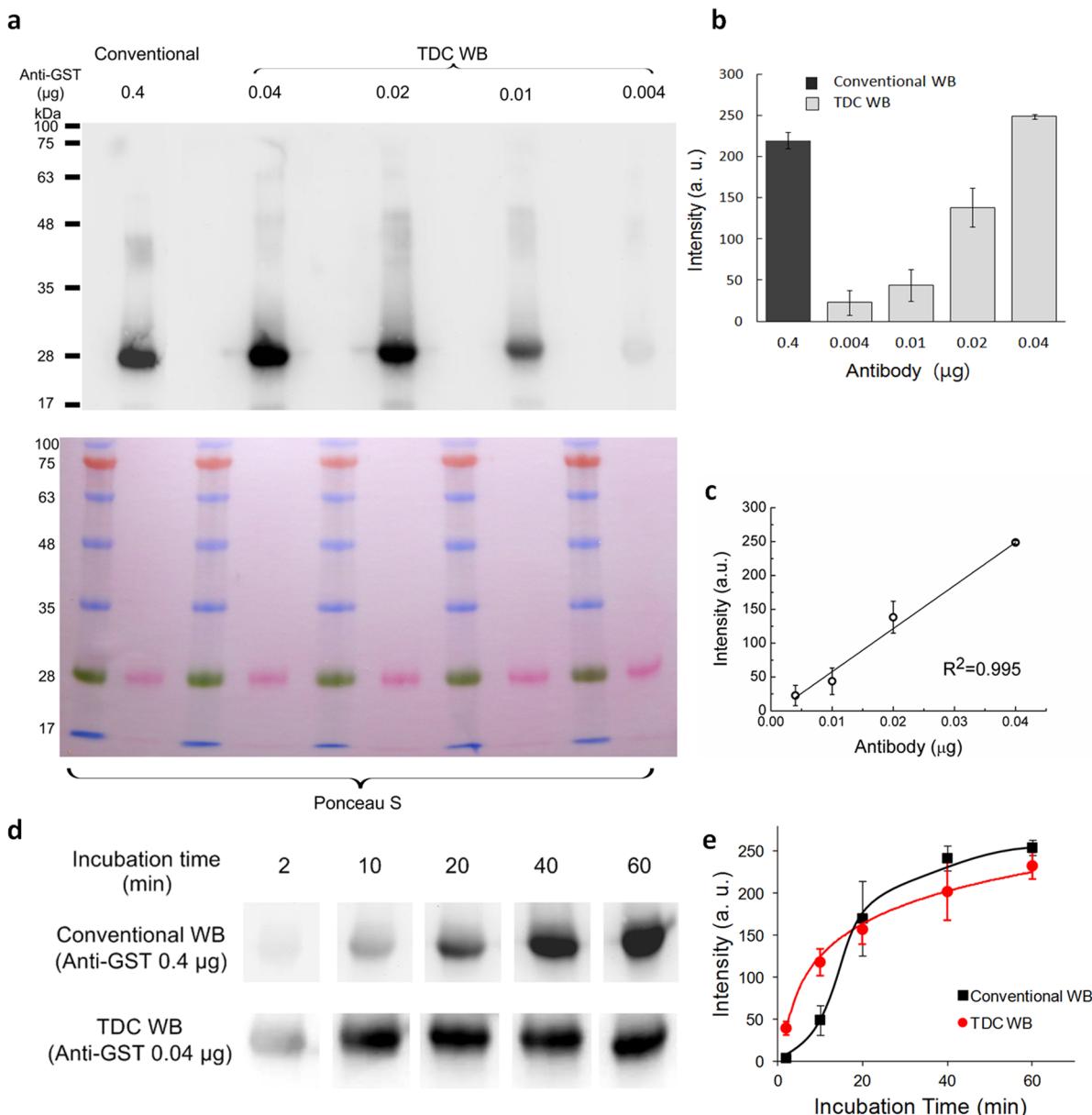
**Protein Expression and Purification.** The expression vector pGEX-4T-3 (GE Healthcare) encoding glutathione S-transferase (GST) was transformed into *E. coli* BL21(DE3) cells (Novagen, EMD Biosciences). Cells were cultured in Luria–Bertani (LB) medium with ampicillin (50 µg/mL) and incubated at 25 °C on an orbital shaker at 150 rpm. Expression of the recombinant GST protein was induced at an  $A_{600}$  of 0.6–0.7 by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.5 mM for 12 h as described previously.<sup>17</sup> GST was purified using a GSTrapTM FF column (GE Healthcare) pre-equilibrated with a binding buffer (20 mM sodium phosphate, 0.15 M NaCl, pH 7.3), and bound proteins were eluted with 20 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0. The protein purity was examined using 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE), and the concentration was determined by the Bradford dye-binding method.<sup>18</sup>

**Cell Lysate Preparation.** HEK-293T cells cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS and 1% Pen-Strep (Gibco, Invitrogen) were lysed in a lysis buffer (50 mM Tris-HCl, pH 7.8, 4% glycerol, 50 mM NaCl, 0.5% NP-40, 1% SDS, 1% Protease Inhibitor Cocktail Set III (EMD Millipore), and 0.1% sodium azide) for 2 h at 4 °C. The supernatant was collected by centrifugation at 20 000g for 10 min at 4 °C. To prepare the *E. coli* cell lysate, cells were cultured in LB medium overnight at 37 °C. Cells were collected by centrifugation at 6 000g for 10 min. The cell pellet was washed three times with PBS and then incubated with a 2× SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.004% bromophenol blue) at 100 °C for 30 min.

**Antibody.** Anti-GST antibody (200 µg/mL) was purchased from Santa Cruz Biotechnology (catalog no. sc-138). Anti-β-actin monoclonal antibody was purchased from Applied Biological Materials (Richmond, BC, Canada; catalog no. G043). Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody (catalog no. G8795) and anti-α-tubulin monoclonal antibody (catalog no. T6074) were purchased from Sigma-Aldrich.

**Gel Electrophoresis.** SDS-PAGE was performed according to the method described by Laemmli.<sup>19</sup> Tris-Glycine SDS running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3) was used as the electrophoresis buffer during the stacking and separation process of SDS-PAGE. After electrophoresis, proteins were transferred onto the PVDF membrane (Immobilon-P, Millipore) at 400 mA for 1 h by using 10 mM CAPS (pH 11) transfer buffer with 15% methanol and then stained with Ponceau S (0.1% (w/v) in 5% acetic acid (Sigma-Aldrich) for rapid reversible detection of protein bands



**Figure 2.** TDC WB exhibited good performance and high-linearity in coating antibody solutions. (a) Purified GST protein ( $6 \mu\text{g}$ ) was analyzed on SDS-PAGE and transferred to a PVDF membrane to perform conventional WB or TDC WB with serial dilutions of anti-GST antibody solutions. The first row shows the immunoblotting results while the second row displays the GST protein stained by Ponceau S. (b) Signal intensity of the experimental results in part a was further measured by densitometer and plotted against the amount of antibody consumption. The error bar indicates the standard deviations of three independent experiments. (c) Detection sensitivity of TDC WB showed high linearity with a 0.999 of correlation coefficient  $R^2$ . (d) Investigation of the effect of the primary antibody incubation time on conventional WB or TDC WB. Purified GST protein ( $6 \mu\text{g}$ ) was analyzed on SDS-PAGE and transferred to a PVDF membrane for incubating with anti-GST antibody in incubation periods ranging from 2 to 60 min. (e) Signal intensity was further measured by a densitometer and plotted against the incubation time. The error bar indicates the standard deviations of three independent experiments.

on the PVDF membrane before conducting the Western blotting analysis.

**Conventional WB.** Conventional WB was performed on an orbital shaker at room temperature. PVDF membranes were blocked with a blocking buffer (5% skimmed milk in PBS with 0.05% Tween-20 (PBST)) for 30 min and then probed with primary antibodies diluted 1:5 000 in 10 mL of PBST for 1 h. The membranes were subsequently washed three times for 10 min each with PBST and then incubated with horseradish peroxidase-conjugated species-specific secondary antibody diluted to 1:5 000 in PBST for 1 h. The membranes were

then washed three times for 10 min each in PBST. VisGlow chemiluminescent substrate (Visual Protein Biotechnology, Taipei, Taiwan) was used to detect the proteins on the membranes, and signals were visualized by the UVP BioSpectrum Imaging System.

**Thin-Film Direct Coating Western Blotting (TDC WB).** Figure 1 shows a schematic diagram of TDC WB. The typical processing stages for Western blotting include gel electrophoresis, transfer, blocking, antibody probing, and analysis. Figure 1a shows comparative schematics for the probing stage for TDC WB (left) and conventional WB (right). In

conventional WB, the blotting membrane was incubated with the primary antibody and continually shaken on an orbital shaker while, in TDC WB, a thin-film of the primary antibody was directly coated on the blotting membrane.

The newly developed thin-film coater<sup>15</sup> (see the schematic diagram in Figure 1a) is made of commercially available standard material, i.e., silicon wafer and glass sheet, and manufactured by using the standard microelectromechanical systems (MEMS). The coater consists of a diffuser with a slit at one end and an interconnector at the other end connected to the reservoir. The diffuser was designed as a fishtail-type structure with an upstream spread angle of 60°. This coater is constructed with a guide-fin in the middle and a contraction at the end of the silicon mold to ensure a uniform flow velocity across the slit width. The diffuser slit is 150 μm in depth and 20 mm in width at the coater exit. The total interior volume of the diffuser is about 80 μL.

The fabrication process of the silicon-based coater has four steps: (i) a silicon substrate was spin coated with 10 mL of photoresist (SPR220, MEGAPOSIT) and baked on a hot plate at 90 °C; (ii) the photoresist on the silicon substrate was patterned and developed via photolithography, which served as the mask for the following etching process; (iii) deep reactive ion etching (DRIE) was used to pattern the silicon substrate. This process of inductively coupled plasma etching produced the vertical side wall of coater; (iv) the patterned silicon substrate was then cleaned to remove the photoresist in heated piranha solution ( $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 = 3:1$  in volume) for 30 min. After these steps, the silicon diffuser was covered by Pyrex glass by anodic bonding, which generated a strong electric field at the interface between the silicon and glass surface to produce a close contact. Facilitated by the strong electric field and high temperature, a hydrogen bond was formed at the interface. The silicon-based coater was then assembled in the holders and fixed to the traversing system as a compact thin-film coating platform.

To prepare the coating process, the PVDF membrane was first placed on a glass plate (6 cm × 8 cm) along the coating direction (Figure 1a) to air-dry excess liquid for 10 min. The primary antibody solution was then fed into the TDC coater using a syringe pump (100 μL/min). The TDC coater was mounted on the vertical axis of a traversing stage to control the coating gap between the coater and the membrane by a self-written LABVIEW program (National Instruments). The stage speed was programmable, and the setting in the study was 1 mm/s. The coating process can be further divided into three steps as shown in Figure 1b. The coater first descended to the surface of the cover glass and fluid pumping began. The coater then moved from the cover glass to a PVDF membrane without direct contact (to PVDF) along the coating direction. When the coating process finished, fluid pumping stopped and the thin-film coater ascended to the resetting position. In the present study, each stroke used less than 0.1 mL (for 20 mm coating width and 60 mm coating length) of the primary antibody solution, and the operation duration was less than a minute (depending on the coating length and speed). After the antibody coating was complete, the PVDF membrane was ready for the subsequent detection and analysis procedures.

## RESULTS AND DISCUSSION

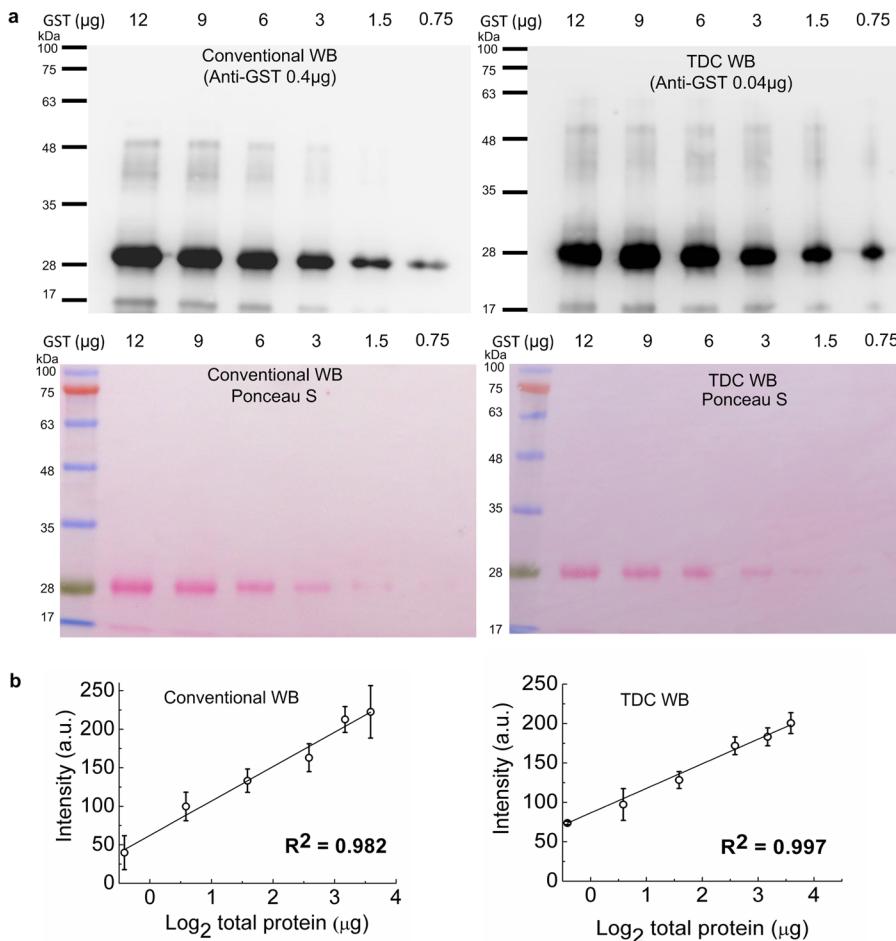
### Application of TDC WB for Analyzing Purified Recombinant GST.

To examine the performance of TDC WB, purified recombinant GST was used as the target protein

for detection by the anti-GST antibody. GST was used for the following reasons: (1) GST and GST fusion proteins are routinely used for studying protein–protein interaction in biochemical experiments; (2) detection of GST by its specific antibody is frequently used in general biochemical laboratories; (3) GST is not sequestered in inclusion bodies while expressing in *E. coli* and usually manifests as a fusion protein to facilitate the proper folding of the GST-fusion protein for protein expression; (4) GST and GST-fusion proteins can be affinity-purified without denaturation; and (5) the specific antibody against GST is commercial available. The purified recombinant GST (6 μg) was analyzed on SDS-PAGE and then transferred to PVDF membranes. Serial dilutions of anti-GST antibody solutions were used to test the performance of TDC WB against that of conventional WB. As shown in Figure 2a, GST was detected by conventional WB with 0.4 μg of anti-GST antibody or by TDC WB with 0.004–0.04 μg of anti-GST antibody, respectively. The detected signal intensity using 0.4 μg of anti-GST antibody on conventional WB was similar to that using 0.04 μg or 0.02 μg of antibody on TDC WB. Figure 2b shows that TDC WB with 0.04 μg of antibody exhibited the highest intensity, with an intensity 12% higher than that of conventional WB with 0.4 μg of antibody. On the basis of this result, unless otherwise mentioned, the PVDF membrane coated with 0.04 μg of antibody was used as the standard condition for TDC WB testing throughout this study. Furthermore, as shown in Figure 2c, TDC WB displayed a very linear relationship of detecting intensity and antibody concentration from a least-squares curve fitting with a root-mean-square error of 0.005 and 0.004–0.04 μg of antibody.

Although the immunoblotting signal in TDC WB obtained using 0.004 μg of antibody in Figure 2a was not visible to the naked eye, it is clearly detectable by a densitometer. The signal intensity can be also easily enhanced either by repeating the same coating process or by using a higher concentration antibody solution (data not shown), indicating that increasing the number of antibody coating layers can proportionally enhance the signal intensity. Therefore, the antibody consumption in TDC WB can be further reduced by using a lower concentration of antibody solution and an optical detector.

In TDC WB, each membrane was only coated once with a layer of thin-film diluted antibody solution (0.4 μg/mL with volume of 0.1 mL) measuring some tens of micrometers in thickness and then statically laid on a glass plate for 1 h to ensure consistent incubation times with conventional WB. Therefore, the interaction between the GST protein and antibody mainly occurs through diffusion within a very small space. In contrast, the membrane blotted with the same amount of GST for conventional WB was incubated with a diluted antibody solution (0.04 μg/mL with volume of 10 mL) some millimeters-deep and continually shaken on an orbital shaker for 1 h. In conventional WB, the interaction between the GST protein and antibody could be enhanced by increasing the number of molecular collisions through the forced convection of the shaking antibody solution. However, the lower antibody concentration and much longer traveling distance for the antibody to bind the GST proteins in the conventional WB restricts effective interaction and reduces detection sensitivity. To quantitatively analyze the influence of these two mechanisms, different experiments were conducted to analyze the incubation time (2–60 min) of the primary antibody in both TDC WB and conventional WB. Figure 2d,e clearly shows



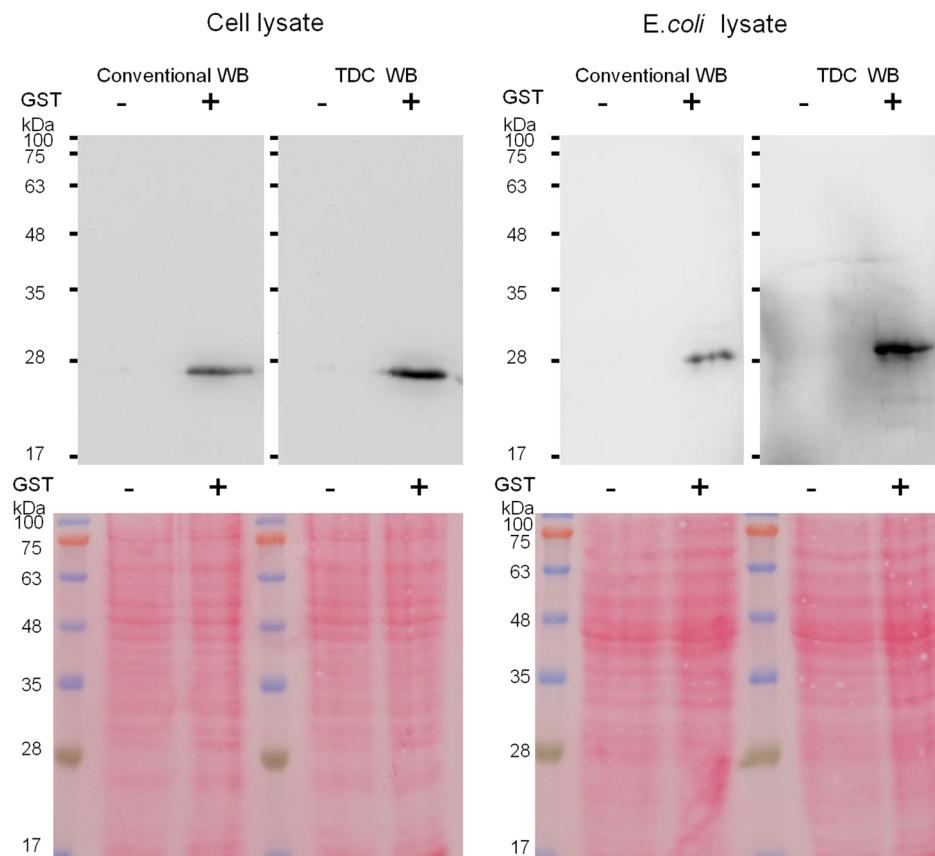
**Figure 3.** Investigation of the detection dynamic range of conventional WB and TDC WB. (a) Various amounts of GST (0.75–12 µg) were analyzed on SDS-PAGE and then transferred to PVDF membranes for detection by conventional WB with 0.4 µg of anti-GST antibody or TDC WB with 0.04 µg of anti-GST antibody. (b) Signal intensity was further measured by a densitometer and plotted against the amounts of GST proteins loaded on SDS-PAGE. Error bars represent the standard deviations of three independent experiments.

that the signal intensity of TDC WB is significantly higher than that of conventional WB at both 2 and 10 min. The signal intensity of conventional WB and TDC WB reached a similar level after 20 min, with the former showing a slightly higher intensity although both curves approach a similar level as time continues to pass. The present results show the diffusion effect dominates a short incubation period and the convection effect starts to enhance the interactions between the GST and anti-GST antibody after the intersection point at around 20 min (Figure 2e). This indicates that the incubation time for TDC WB can be significantly reduced from 60 to 10 min. On the basis of this diffusion mechanism, reducing the thickness of the coating film and increasing the antibody concentration should shorten the incubation time. In this study, the incubation time was kept 60 min in accordance with the general protocol for conventional WB. Further studies for optimizing operation conditions are still needed.

**Linear Characteristic of TDC WB for Protein Quantification.** To determine whether the signal intensity of TDC WB was directly proportional to the changes in protein sample concentrations used in the experiments, a series of purified GST samples from 0.75 µg to 12 µg was analyzed using SDS-PAGE with conventional WB or TDC WB. The results showed that the signal intensity obtained from TDC WB (Figure 3a, lower panel) was slightly stronger than that obtained from

conventional WB (Figure 3a, upper panel). The immunoblotting results in Figure 3a were further measured by densitometry and are shown in Figure 3b, revealing a linear 4-log<sub>2</sub> dynamic range for GST measurement using TDC WB. The signal intensity was plotted against the amount of total protein used in the experiment. The correlation coefficient  $R^2$  obtained by using conventional or TDC WB was 0.982 or 0.997, respectively. In short, TDC WB is found to be a reliable method for protein quantification which provides material cost savings of up to 3/4 (see cost analysis in the Supporting Information) and operation time.

**Detection of Target Proteins in Cell Lysates by TDC WB.** In addition to analyzing the purified GST protein in a cell-free system, TDC WB was also used to detect extrinsic GST proteins added in crude cell lysate such as 293T or *E. coli* cell lysate (Figure 4). The immunoblotting membranes stained with Ponceau S showed a complicated protein composition in the cell lysate (lower panels in Figure 4a,b). Moreover, the extrinsic GST proteins (6 µg) added in the indicated samples cannot be clearly recognized by Ponceau S staining. However, after performing conventional WB or TDC WB, extrinsic GST was successfully detected (upper panels in Figure 4a,b) and the signal intensity obtained from TDC WB is approximately twice as strong as that from conventional WB.



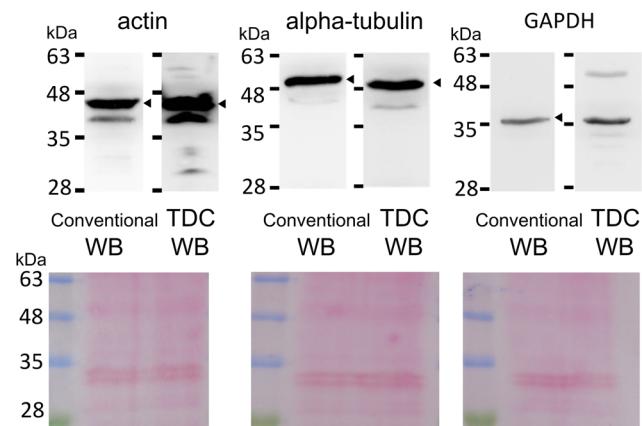
**Figure 4.** TDC WB specifically detected extrinsic GST proteins in cell lysates. GST ( $6\ \mu\text{g}$ ) was mixed with  $6\ \mu\text{g}$  of 293T cell lysate (a) or *E. coli* cell lysate (b) and then analyzed on SDS-PAGE. The cell lysate without adding GST was also analyzed as the negative control. Electrophoresis was calibrated using prestained SDS-PAGE molecular weight standards as indicated in the figure (Geneaid Biotech, Taipei, Taiwan). The data shown here are representative of three independent experiments.

To further investigate whether TDC WB can specifically detect intrinsic proteins in 293T cells, two cytoskeleton proteins ( $\beta$ -actin and  $\alpha$ -tubulin) and one cytosolic enzyme GAPDH were also analyzed using individual specific antibodies. These three proteins are widely used in biochemical experiments as so-called “internal controls” or “loading controls”. The data in Figure 5 show that TDC WB performs as well as conventional WB in detecting intrinsic proteins in crude cell lysate.

Using TDC WB, minimal amounts of the specific primary antibody can be homogeneously coated on the blotting membrane. Using a 20 mm-wide thin film coater, only  $0.04\ \mu\text{g}$  of the anti-GST antibody was used to coat a  $10\ \text{mm} \times 60\ \text{mm}$  PVDF membrane. Clearly, antibody consumption can be further reduced by using a narrower coater. Figure 6 shows the results of TDC WB using coaters with channel exit widths of 20, 1, 0.1, and 0.02 mm. All tests produced consistent results and indicate the antibody consumption can be further reduced from  $4 \times 10^{-2}$  to  $4 \times 10^{-5}\ \mu\text{g}$  with the same coating setting parameters by using a 0.02 mm coater rather than a 20 mm one. This means that the antibody consumption in TDC WB could be further reduced by 4 orders of magnitude less than that needed in conventional WB.

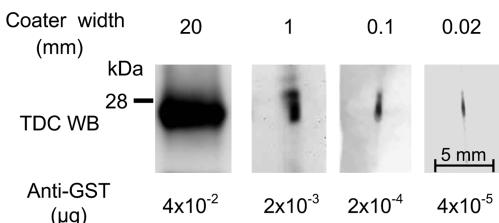
## CONCLUSIONS

Thin-film direct coating (TDC) technology successfully applied to Western blotting (WB) with a 4-log<sub>2</sub> dynamic detection range without significantly changing experimental preparation



**Figure 5.** TDC WB performed well in detecting intrinsic proteins. The cell lysate of 293T cells ( $6\ \mu\text{g}$ ) was analyzed on SDS-PAGE and then subjected to conventional WB or TDC WB as described previously.  $\beta$ -actin,  $\alpha$ -tubulin, or GAPDH was detected by its specific antibody as indicated. The data shown here are representative of three independent experiments.

processes. TDC WB allows the specific primary antibody to be efficiently and homogeneously coated on the blotting membrane with very low consumption of test materials with a much shorter incubation time. For instance, the antibody consumption of TDC WB can be easily and dramatically reduced from  $10^{-1}$  to only  $10^{-4}$  of the amount originally used in conventional WB by simply reducing the coating width.



**Figure 6.** TDC WB with different coating widths. Purified GST protein ( $6\ \mu\text{g}$ ) was analyzed on SDS-PAGE and transferred to a PVDF membrane for TDC WB by different coaters with channel exit widths of 20, 1, 0.1, and 0.02 mm. Anti-GST antibody consumption amounts are shown in the bottom row.

Similarly, applying TDC WB reduces probing time from 60 to 10 min or less. This programmable and user-friendly coating platform could be easily integrated into routine Western blotting analyses in general biochemical and medical diagnostic laboratories.

## ■ ASSOCIATED CONTENT

### S Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

<sup>§</sup>Y.-K.Y. and Y.-W.J. contributed equally to this work.

### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Dechend, R.; Homuth, V.; Wallukat, G.; Muller, D. N.; Krause, M.; Dudenhausen, J.; Haller, H.; Luft, F. C. *J. Soc. Gynecol. Invest.* **2006**, *13*, 79–86.
- (2) Peter-Katalinic, J. *Methods Enzymol.* **2005**, *405*, 139–171.
- (3) Sakudo, A.; Suganuma, Y.; Kobayashi, T.; Onodera, T.; Ikuta, K. *Biochem. Biophys. Res. Commun.* **2006**, *341*, 279–284.
- (4) Westermeier, R.; Marouga, R. *Biosci. Rep.* **2005**, *25*, 19–32.
- (5) Hughes, A. J.; Herr, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 21450–21455.
- (6) Flisiak, R.; Wierzbicka, I.; Prokopowicz, D. *Roczn. Akad. Med. Białystok* **1998**, *43*, 210–220.
- (7) Egger, D.; Bienz, K. *Mol. Biotechnol.* **1994**, *1*, 289–305.
- (8) Anderson, G. J.; Cipolla, C. M.; Kennedy, R. T. *Anal. Chem.* **2011**, *83*, 1350–1355.
- (9) Millipore Corporation Home Page. <http://www.millipore.com/techlibrary/index.do> (accessed April 6, 2013).
- (10) O'Neill, R. A.; Bhamidipati, A.; Bi, X. H.; Deb-Basu, D.; Cahill, L.; Ferrante, J.; Gentalen, E.; Glazer, M.; Gossett, J.; Hacker, K.; Kirby, C.; Knittle, J.; Loder, R.; Mastroeni, C.; MacLaren, M.; Mills, T.; Nguyen, U.; Parker, N.; Rice, A.; Roach, D.; Suich, D.; Voehringer, D.; Voss, K.; Yang, J.; Yang, T.; Van der Horn, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 16153–16158.

- (11) He, M.; Herr, A. E. *Anal. Chem.* **2009**, *81*, 8177–8184.
- (12) He, M.; Herr, A. E. *J. Am. Chem. Soc.* **2010**, *132*, 2512–2513.
- (13) Pan, W.; Chen, W.; Jiang, X. *Anal. Chem.* **2010**, *82*, 3974–3976.
- (14) Kistler, S. F.; Schweizer, P. M. *Liquid Film Coating*, 1st ed.; Chapman & Hall: London, 1997.
- (15) Wang, A.-B.; Hsieh, Y.-W.; Liu, Y.-J. *Coating Module*. U.S. Patent Application 20140000511, January 2, 2014.
- (16) Wang, A. B.; Hsieh, Y. W.; Liu, Y. J.; Lin, T. Y. Could a Die Be Disposable? - Design and Test of a Silicon-Wafer-Based Slot Die Coater. In *Proceedings of the 16th International Coating Science and Technology Symposium*, Midtown Atlanta, Georgia, September 9–12, 2012.
- (17) Shin, Y. C.; Tang, S. J.; Chen, J. H.; Liao, P. H.; Chang, S. C. *PLoS One* **2011**, *6*, No. e27742.
- (18) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
- (19) Laemmli, U. K. *Nature* **1970**, *227*, 680–685.