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APPLICATION NOTE

SIMPLE WESTERN ASSAYS FOR DETECTION OF ACE2 AND TMPRSS2, KEY PLAYERS IN SARS-COV-2 INFECTION

INTRODUCTION TO MECHANISM OF SARS-COV-2 INFECTION

In December of 2019, an easily transmissible and deadly novel coronavirus emerged called SARS-CoV-2 (COVID-19), causing a global pandemic and a socio-economic crisis. Currently, our understanding of SARS-CoV-2 entry into host cells involves the binding of the viral spike protein to the human ACE2 receptor through the spike protein's receptor-binding domain (RBD). Then, the spike protein is cleaved by human protease TMPRSS2 at the S1/S2 boundary or within the S2 subunit. This cleavage removes the structural constraint of S1 on S2, initiating the fusion of the viral and cellular membranes. Finally, the viral genome enters the host cell and infection begins (**Figure 1**).[1,2]

An important part of understanding SARS-CoV-2 infection is the detection and quantification of proteins involved in viral binding and activation. For example, the density of ACE2 and TMPRSS2 in host tissue could be a measure for infection susceptibility because of their key roles in SARS-CoV-2 infection. Therefore, being able to detect and even change ACE2 and TMPRSS2 expression will lead to a better understanding of the disease and possibly a mode of preventing infection.

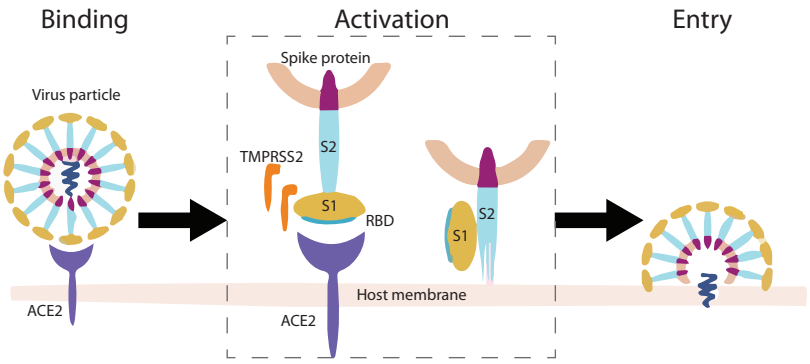

FIGURE 1. Schematic of SARS-CoV-2 binding, activation, entry and uncoating. The virus particle contains spike protein composed of S1 and S2 subunits on its surface. The spike protein binds to the host ACE2 receptor. The host protease TMPRSS2 cleaves the spike protein, activating viral entry and uncoating.

THE SIMPLE WESTERN™ ADVANTAGE

Simple Western from ProteinSimple is a fully automated, capillary-based open platform to generate Western blot data. It has many advantages over traditional Western blot, including:

- Fast time to results (3-hour run times)
- Built-in analysis software that allows for immediate quantitation
- Flexible throughput with up to 96 samples analyzed per run
- Excellent reproducibility due to the exclusion of all manual steps
- Any commercial or custom antibody may be used for target protein detection
- Small sample volume requirement (3 µL), and up to 8 data points can be generated out of just 5 µL
- With the new RePlex™ feature on Jess™, you can run two immunoassays within the same capillary
- Perhaps most importantly given the current climate of social distancing, Simple Western may be run and analyzed remotely, minimizing time in the laboratory.

SIMPLE WESTERN CAN BE DEPLOYED FOR COVID-19 RESEARCH

In this Application Note, we demonstrate how Simple Western can be applied to the detection and characterization of ACE2 and TMPRSS2, key players in SARS-CoV-2 infection. As an open platform, any commercial or custom antibody may be used for target protein detection on Simple Western. Here, we used an anti-ACE2 antibody from R&D Systems and an anti-TMPRSS2 antibody from Novus Biologicals to develop immunodetection assays for these proteins in purified form and expressed in human cells. This resulted in assays that were highly sensitive over a large dynamic range, providing molecular weight information and even quantification of ACE2 and TMPRSS2 in human cells.

ANALYZING COVID-19 INFECTION USING SIMPLE WESTERN SYSTEMS

The reagents used in this study are listed in **Table 1**.

Samples were prepared according to the instructions listed in the 12-230 kDa Peggy Sue or Sally Sue Separation Module. The anti-ACE2 and anti-TMPRSS2 antibodies were diluted 1:50 (a concentration determined to be at saturation for both targets) in Milk-Free diluent and detected with an anti-goat secondary antibody. It should be noted that for antibodies raised in goat, the Milk-Free Antibody Diluent should be used to reduce background noise. For more information, see our technical note A Simple Way to Reduce Background Signal Associated with Anti-Goat Secondary Antibodies. Following sample and antibody preparation, all steps were performed automatically by Peggy Sue™, a Simple Western Instrument from ProteinSimple.

Peggy Sue from ProteinSimple

REAGENTS USED IN THIS STUDY		
NAME	VENDOR	PART NUMBER
Anti-Goat Detection Module for Wes, Peggy Sue and Sally Sue	ProteinSimple	DM-006
12-230 kDa Peggy Sue or Sally Sue Separation Module	ProteinSimple	SM-S001
Human/Hamster ACE-2 Antibody	R&D Systems	AF933
Recombinant Human ACE-2 Protein, CF	R&D Systems	933-ZN-010
Human Kidney Whole Tissue Lysate (Adult Whole Normal)	Novus Biologicals	NB820-59231
Recombinant Human TMPRSS2 GST (N-Term) Protein	Novus Biologicals	H00007113-Q01
TMPRSS2 Antibody	Novus Biologicals	NBP1-20984
TMPRSS2 Overexpression Lysate	Novus Biologicals	NBL1-17121

TABLE 1. Reagents used in this study. The anti-ACE2 and anti-TMPRSS2 antibodies were raised in goat and detected using the Anti-Goat Detection Module.

To develop an assay for ACE2 detection and quantification, we created a 3-fold dilution series of the recombinant ACE2 protein from 1 ng/mL to 0 pg/mL, and then we analyzed this dilution series on Simple Western with the anti-ACE2 antibody. This analysis resulted in a single, well-defined peak corresponding to ACE2. As expected, the signal decreased with decreasing sample concentration (**Figure 2A-2B**). To calculate the linearity of detection in this assay, we plotted the peak area of ACE2 by concentration. This analysis resulted in a highly linear trend with an R^2 value of 0.994 (**Figure 2C**). To demonstrate the specificity of the assay, we analyzed human whole kidney tissue lysate. When the sample was loaded at a concentration of 13 µg/mL on Peggy Sue, a well-defined peak corresponding to ACE2 appeared, with minimal cross-reactivity between the anti-ACE2 antibody and host proteins (**Figure 2D**). Using the standard curve generated in Figure 2C, we calculated that in 13 µg/mL of lysate, there was 0.5453 ng/mL of ACE2. Collectively, these result demonstrate that Simple Western could detect and quantify ACE2 with high sensitivity and specificity over a large dynamic range.

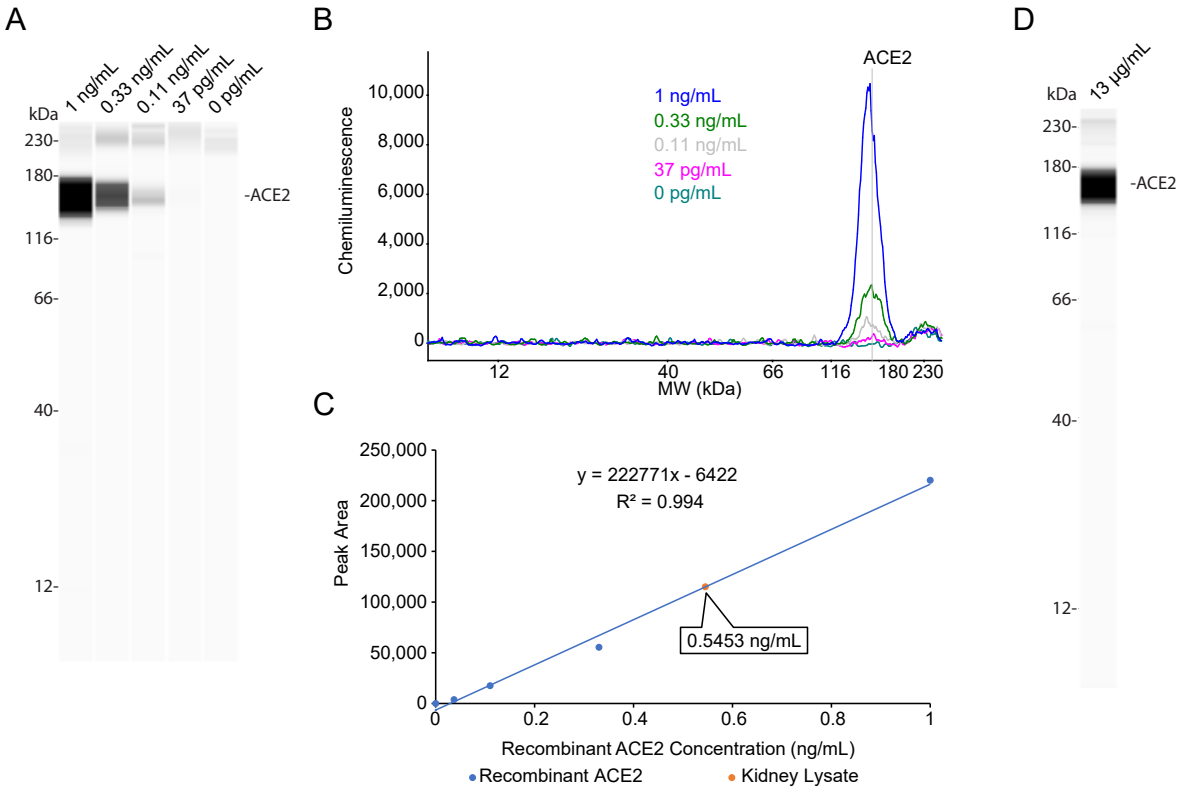

FIGURE 2. A Simple Western assay for ACE2 detection. (a) Lane view of a serial dilutions series of recombinant ACE2 protein detected with an anti-ACE2 antibody. (b) Electropherogram of the serial dilution series of the recombinant ACE2 protein detected with an anti-ACE2 antibody. (c) The peak area of the ACE2 signal plotted against the concentration of recombinant ACE2 protein. The line represents a linear trendline with a its equation and R^2 value shown. The data point in orange represents the calculated concentration of ACE2 in 13 µg/mL of kidney lysate shown in panel d using the linear equation provided. (d) Kidney lysate was analyzed on Simple Western at a concentration of 13 µg/mL and detected with an anti-ACE2 antibody.

To develop an assay for TMPRSS2 detection and quantification, we created a 3-fold dilution series of the recombinant TMPRSS2 protein from 0.5 µg/mL to 0.3 ng/mL, and then we analyzed this dilution series on Simple Western with the anti-TMPRSS2 antibody. This analysis resulted in a single, well-defined peak corresponding to TMPRSS2. As expected, the signal decreased with decreasing sample concentration (**Figure 3A-3B**). To calculate the linearity of detection in this assay, we plotted the peak area of TMPRSS2 by concentration. This analysis resulted in a highly linear trend with an R^2 value of 0.999 (**Figure 3C**). To demonstrate the specificity of the assay, we analyzed HEK293 cells overexpressing TMPRSS2. When the sample was loaded at a concentration of 37 µg/mL on Peggy Sue, a well-defined peak corresponding to TMPRSS2 appeared, with minimal cross-reactivity between the anti-TMPRSS2 antibody and host proteins (**Figure 3D**). Using the standard curve generated in Figure 3C, we calculated that in 37 µg/mL of lysate, there was 25.32 ng/mL of TMPRSS2. Collectively, these result demonstrate that Simple Western could detect and quantify TMPRSS2 with high sensitivity and specificity over a large dynamic range.

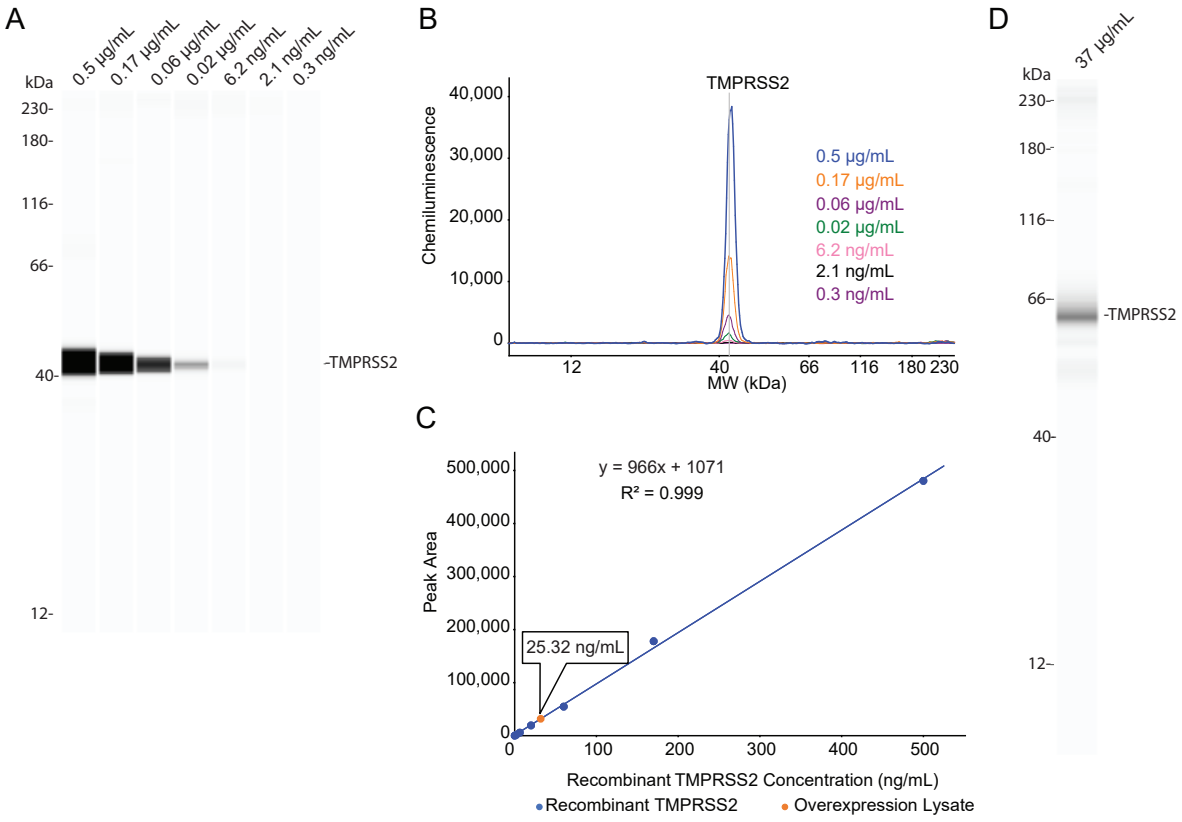

FIGURE 3. A Simple Western assay for TMPRSS2 detection. (a) Lane view of a serial dilutions series of recombinant TMPRSS2 protein detected with an anti-TMPRSS2 antibody. (b) Electropherogram of the serial dilution series of the recombinant TMPRSS2 protein detected with an anti-TMPRSS2 antibody. (c) The peak area of the TMPRSS2 signal plotted against the concentration of recombinant TMPRSS2 protein. The line represents a linear trendline with a its equation and R^2 value shown. The data point in orange represents the calculated concentration of ACE2 in 37 µg/mL of overexpression lysate shown in panel d using the linear equation provided. (d) A lysate of HEK293 cells overexpressing TMPRSS2 was analyzed on Simple Western at a concentration of 37 µg/mL and detected with an anti-TMPRSS2 antibody.

IMMUNOASSAYS TO STUDY COVID-19

Here, we developed immunoassays on Simple Western for detection and quantification of ACE2 and TMPRSS2, key players in SARS-CoV-2 infection resulting in COVID-19. Detection of these proteins with Simple Western was highly sensitive, with low ng-level sensitivity for recombinant TMPRSS2 and in the pg-level for recombinant ACE2. The dynamic range of these assays was highly linear, with R^2 values >0.994 for each assay. Unlike related immunoassay methods like ELISA and FACS, Simple Western provided molecular weight information in addition to quantification. This allows for more complete characterization of your target, including aggregated and degraded forms of the protein that have different molecular weights that cannot be discerned by other techniques. Ultimately, the large and highly linear dynamic range of detection allowed for the calculation of ACE2 and TMPRSS2 density in a human cells. In 13 µg/mL of human kidney lysate, a density of 0.5453 ng/mL of ACE2 was calculated, and in 37 µg/mL of overexpression lysate, approximately 25.32 ng/mL of TMPRSS2 was calculated. The ability to calculate ACE2 and TMPRSS2 density in host cells can shed valuable insight into SARS-CoV-2 infection susceptibility.

These assays were quick and easy to develop, and the automation Simple Western provided made it possible to run and analyze experiments remotely following a simple sample preparation. This advantage is critical in the era of mandatory social distancing because it brings the amount of time needed to spend in the laboratory to a minimum. For information on how to save and access Simple Western data remotely, see our protocol on Remote Tools for Simple Western.

REFERENCES

1. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. jin, J. Wang, and Z. Qian, *Nature Communications*, 2020; **11**(1620).

2. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N. Wu, A. Nitsche, M.A. Müller, C. Drosten, and S. Pöhlmann, *Cell*, 2020; **181**: 271-280.

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