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Generation of Recombinant Antibodies in HEK293F Cells for the Detection of *Staphylococcus aureus*

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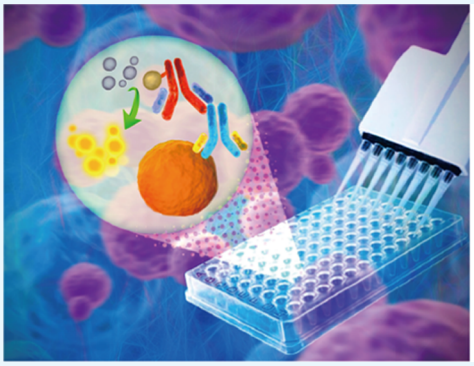

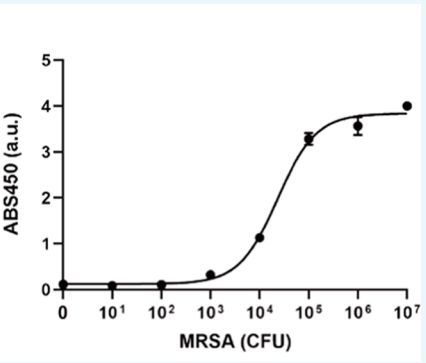

ABSTRACT: *Staphylococcus aureus* is a major resistant pathogen in clinical practice. Due to the increasing number of infections, rapid and sensitive detection of antibiotic-resistant *S. aureus* as well as antibiotic-sensitive *S. aureus* is important for the prevention and control of infectious diseases. In this study, we produced recombinant antibodies against *S. aureus* from mammalian human embryonic kidney 293 Freestyle cells with high yield and purity. These recombinant antibodies showed high binding affinity and low detection limit in both indirect and sandwich enzyme-linked immunosorbent assays for the detection of methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus*. These results suggest that the recombinant antibodies produced herein can be used for the accurate detection of *S. aureus* with a wild range of applications in medical diagnosis, food safety, and drug discovery.

1. INTRODUCTION

Staphylococcus aureus is a widely distributed pathogen that causes many infectious diseases, such as septicemia, osteomyelitis, pneumonia, toxic shock syndrome, and endocarditis.[1,2] The current treatment of *S. aureus* infection relies on antibiotics, but highly antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), are an increasing public health risk. Thus, the development of rapid, sensitive, and exact quantitation methods for detecting both methicillin-sensitive *S. aureus* (MSSA) and MRSA is vital for medical diagnosis, food safety, environmental hygiene, and drug discovery. To date, a culture and colony-counting-based method is standard for detecting *S. aureus*.[3] However, practical applications of this conventional method are limited because it requires 3−5 days to obtain the results, which is time-consuming and inadequate for timely *in situ* detection. Polymerase chain reaction (PCR)-based detection is alternatively used in current practice, but it still requires 3−4 h, and the specificity is low.[4] The qPCR-based method has low selectivity even though the limit of detection (LOD) is 10^3 CFU/mL.[5] Other molecular diagnostics using lectin or aptamer combined with flow cytometry have been developed to detect *S. aureus*.[6,7] However, these methods suffer from low selectivity and labor intensity.

Immunological assays have been developed for detecting *S. aureus* with high accuracy because of the high specific response of antibody to antigen. For example, the surface plasmon resonance (SPR) detects *S. aureus* in a label-free and real-time manner with a LOD of 10^3−10^4 CFU/mL.[8,9] However, this method requires phage production, isolation, and purification, which have limitations due to complicated handling. Subramanian et al. performed SPR using an antibody against protein A (PA), which is expressed on the surface of *S. aureus*, but the LOD of the system was high (10^7 CFU/mL).[10] Additionally, as the SPR-based assay needs sensor chips, the technology needs a high cost. A quartz crystal microbalance

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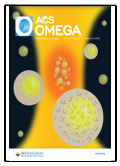

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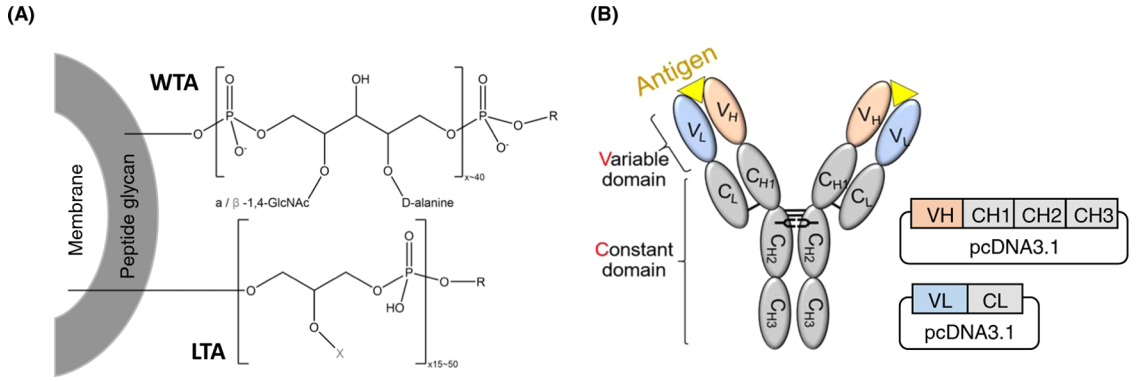

Figure 1. (A) Schematic representation of WTA and LTA on *S. aureus*. (B) Schematic images of the full-sized antibody (left) and plasmid DNA maps for the expression of the H chain and L chain of recombinant antibody (right).

with a random antibody shows high specificity for real-time detection of *S. aureus*.[11] However, it requires control over antibody orientation to ensure Fc recognition and the response affected by the antibody content of the sample.

Enzyme-linked immunosorbent assay (ELISA) can be used for *S. aureus* detection because of its simplicity, low cost, and reliability. However, as conventional ELISA-based *S. aureus* detection uses hybridoma-based antibodies, the high cost of obtaining antibodies is a major burden.[12−14] In addition, hybridoma clones, which are used for producing monoclonal antibodies, lose their secreting ability over time.

A recombinant antibody has attractive attributes compared to the traditional hybridoma-based antibody: (1) it can be overexpressed with high yield, and its production is controlled; (2) it is expressed from a unique gene, so the sequence information allows for subcloning to be modified or to generate fusions with other molecules, such as fluorescent proteins, enzymes, and peptides, without interfering with their antigen-binding properties; and (3) several issues with hybridoma production, such as gene mutations and cell line drift, can be avoided, leading to a high level of consistency between batches. *Escherichia coli* is one of the systems for producing recombinant proteins including the antibody. To produce a functional antibody using *E. coli*, the key to allow the correct formation of disulfide bonds is the secretion of V chains into the periplasmic space of *E. coli*, the oxidizing environment, for assembling into a functional Fv fragment.[15] Therefore, the production of recombinant antibodies using *E. coli* sometimes results in nonfunctional aggregates, and the recovery of functional antibody from inclusion bodies by denaturation followed by refolding is difficult and time-consuming.[16] Small antibody fragments, such as the single chain fragment of variable domain (scFv) and the antigen-binding fragment (Fab), can be relatively readily expressed in *E. coli*. However, the yield of a larger full-sized antibody in soluble form is generally low because it requires post-translational modifications (PTMs). This has led to the development of a mammalian expression system.

Eukaryotic cells, including mammalian cells, have developed an advanced folding, PTM, glycosylation, and secretion apparatus compared with bacteria.[17] Moreover, antibodies from mammalian cells are indistinguishable from those in humans, with least concerns for immunogenic modifications. Human embryonic kidney (HEK)293 cells and Chinese hamster ovary (CHO) cells are commonly used for producing a high yield of protein that is hard to be expressed in bacteria. HEK293 cells, human-derived cell line, provide the best environment for the expression of human antibodies, and cell lines derived from other species, such as CHO cells, have been found to introduce nonnative PTMs not present on human antibodies.[18] These foreign PTMs can cause immunological reactions in humans and may affect the stability and function of recombinant antibodies.[18] Moreover, HEK293 cells can be transfected with recombinant plasmids for antibody production.[19−22] A highly useful derivative of HEK293 cells has been the establishment of HEK293Freestyle (HEK293F) cells, which are a variant of HEK293 cells and have been widely used to produce challenging proteins.[19] The HEK293F cell line has been adapted to suspension growth, and transient antibody expression allows convenient production.[23] Thus, HEK293F cells can offer a rapid method to produce large quantities of recombinant antibodies. In addition, it has been known that HEK293F cells show high transfection efficiency with the use of the inexpensive polymeric reagent polyethylenimine (PEI), and the results can be evaluated within 48 h after transient transfection, which provides a fast and economical alternative to produce recombinant antibodies.[24−26]

In this study, we generated novel recombinant antibodies against *S. aureus* through mammalian cell culture. We produced large amounts of antibodies from HEK293F cells and purified them in a convenience manner. We revealed the binding efficiency of the produced antibodies to MSSA and MRSA by performing both indirect ELISA and sandwich ELISA, indicating the usefulness of these antibodies for sensitive detection of *S. aureus*.

2. RESULTS AND DISCUSSION

2.1. Construction of Antibody Expression Genes. *S. aureus* incorporates peptidoglycans and membrane-attached cell envelope-associated glycopolymers, such as wall teichoic acid (WTA) and lipoteichoic acid (LTA). WTA on *S. aureus* is composed of *N*-acetylmannosamine (ManNAc)-(α/β-1,4)-*N*-acetylglucosamine (GlcNAc) followed by 11−40 ribitol phosphate (RboP) repeating units (Figure 1A).[27] Lupardus's group bacterially produced three Fabs, 6DWI, 6DW2, and 6DWC, that specifically recognize β-1,4-GlcNAc of WTA in B-cells derived from patients recovering from *S. aureus* infection and revealed their sequences, 3D structures, and antigen-binding efficiencies against MRSA strain USA 300 (KD < 10 nM).[28] However, the detailed production method as well as the purity and yield of the generated antibodies are not shown. Moreover, as the binding efficiency of antibodies was examined

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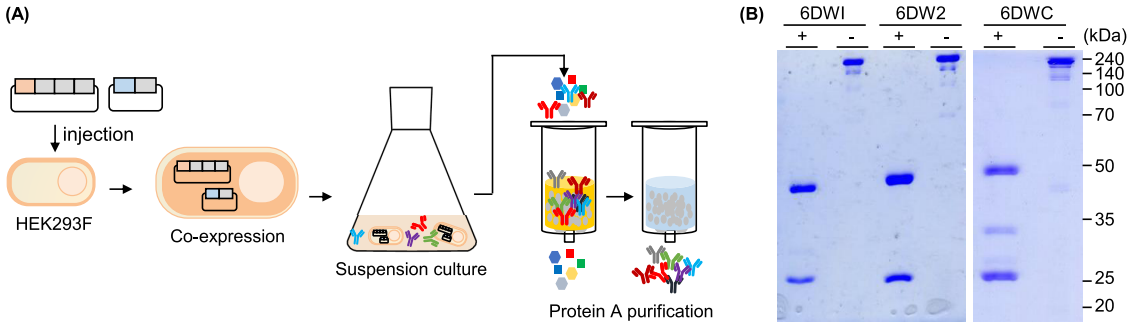

Figure 2. (A) Schematic representation of HEK293F-based recombinant anti-*S. aureus* antibody generation. (B) SDS-PAGE analysis of 6DWI, 6DW2, and 6DWC antibodies. + and − indicate reduced proteins by heating and adding DTT and nonreduced proteins without heating and DTT, respectively. HEK, human embryonic kidney; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and DTT, dithiothreitol.

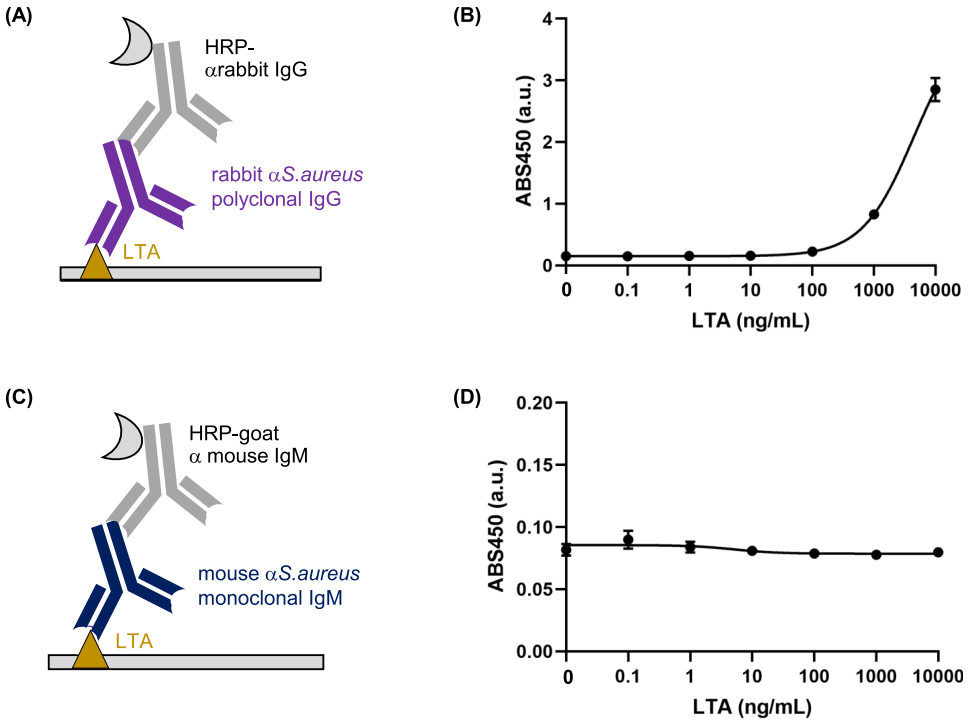

Figure 3. LTA-binding efficiency of commercial antibodies. (A) Schematic representation of indirect ELISA for confirming the dose-dependent LTA-binding efficiency of the polyclonal anti-*S. aureus* antibody. (B) ELISA signal of the polyclonal antibody at different concentrations of LTA. (C) Schematic representation of indirect ELISA for confirming the dose-dependent LTA-binding efficiency of the monoclonal anti-*S. aureus* antibody. (D) ELISA signal of the monoclonal antibody at different concentrations of LTA. Error bars represent ±1 SD (n = 3).

using one MRSA strain, other *S. aureus* strains were required to be used as target antigens to enlarge the versatility of the antibodies. To this end, we aimed to produce large amounts of antibodies against MRSA and MSSA and to describe the information about generating the antibodies as well as their application as ELISA reagents. Particularly, we changed the type of antibody from Fab to full-size immunoglobulinG (IgG) and the host cell from *E. coli* to mammalian cells to improve the function and production yield of the antibodies.

In this study, we constructed three anti-WTA IgG-encoding genes and expressed the recombinant antibodies using a HEK293F cell-based expression system. Afterward, we conducted both indirect and sandwich ELISAs. We confirmed the binding efficiency of the recombinant antibodies to MRSA and MSSA, and then compared the affinity and sensitivity to those of commercial antibodies. First, based on the sequences of the three Fabs, 6DWI, 6DW2, and 6DWC,[28] we codon-optimized VH and VL of each Fab and then separately introduced each synthesized DNA to the pcDNA3.1(−) antibody expression vector (Figure 1B). We added the signal peptide (caccatgggatggagctgtatcatcctcttcttggtagcaacagcta-caggtgtacactcc) at the N-terminal of the heavy (H) or light (L) chain, the GCGGCCGC sequence in front of the signal peptide for inserting the *Not*I site, and the TAAAAGCTT sequence at the C-terminal of the H or L chain for inserting the *Hind*III site. In the case of the H chain expression vector, the GAATTC sequence was added between CH1 and Fc (IgG4) for inserting the *Eco*RI site. We prepared the plasmid with 1 mg/mL concentration with high purity for effective transient co-transfections using PEI.

2.2. Expression and Purification of Antibodies. We expressed the full-size antibodies in HEK293F cells because the cell lines can be efficiently transfected with plasmid DNA on a large scale by PEI and they have been widely used for

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transient protein expression in a serum-free suspension culture. We injected the DNA to the cells and performed a suspension culture. After that, we purified the supernatant with the use of PA beads, which primarily bind to the Fc region of the antibody, in addition to between the CH2 and CH3 domains. Afterward, we changed the elution buffer to phosphate-buffered saline (PBS) using ultrafiltration for desalting (Figure 2A). Finally, we performed size-exclusion chromatography (SEC). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses of the purified supernatant revealed major bands corresponding to the H and L chains of the antibodies with a molar mass of approximately 50 and 25 kDa, under reducing conditions, without heating and adding dithiothreitol (DTT), and full-size antibodies at approximately 150−250 kDa under native conditions (Figure 2B). Based on the amino acid sequences, the expected sizes of the H and L chains of 6DWI, 6DW2, and 6DWC were 116 and 58 kDa for 6DWI, 118 and 26 kDa for 6DW2, and 116 and 60 kDa for 6DWC, respectively, which is consistent with SDS-PAGE results. This result indicates successful expression and folding of the antibodies. We measured the concentrations of purified antibodies using nanodrop, resulting in 200−600 μg of antibodies per 100 mL of the HEK293F culture.

2.3. Binding Efficiency to LTA on the Surface of *S. aureus*. Prior to the confirmation of the antigen-binding activity of recombinant antibodies, we performed ELISA using commercial anti-*S. aureus* antibodies to optimize an ELISA system. As Lupardus's group revealed that the three Fabs showed efficient WTA binding,[28] we first tried to use WTA as an antigen for confirming the binding activity of antibodies. However, as WTA was not commercially available, we used LTA as an antigen instead. Among the four types of LTA, LTA type I of *S. aureus* is composed of 1,3-polyglycerol-phosphate repeating units that are substituted at the C2 position (X in Figure 1A) with the hydrogen proton (−15%), D-alanyl ester (−70%), or *N*-GlcNAc (−15%) (Figure 1A). Although LTA is anchored to the cell membrane, WTA is covalently linked to peptidoglycan, their constructs are structurally similar and both are *N*-GlcNAc-modified.[29] Therefore, we estimated that the three recombinant antibodies, whose sequences were from Fabs against β-1,4-GlcNAc as well as commercial anti-*S. aureus* antibodies, had binding affinity to LTA.

To this end, we performed indirect ELISA using LTA as an antigen, commercial anti- *S. aureus* antibodies, rabbit anti-*S. aureus* polyclonal IgG, or mouse anti-*S. aureus* monoclonal IgM, as a primary antibody, and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG or HRP-conjugated anti-mouse IgM as a secondary antibody (Figure 3). The median effective concentration (EC50) of the polyclonal antibody was $4.49 \pm 0.64 \times 10^3$ ng/mL whereas EC50 of the monoclonal antibody was not detectable (Table 1). These results indicated that the commercial polyclonal antibody can be used for detecting LTA in the ELISA system. This information was revealed for the first time in this study, whereas the detailed experimental conditions had not been exactly presented in the commercial information. As expected, the antigen-binding efficiency of the polyclonal antibody was superior to the one of the monoclonal antibody. Generally, as a monoclonal antibody recognizes a single epitope of an antigen, its affinity and selectivity to the target antigen are higher than those of a polyclonal antibody. The two commercial antibodies were generated by immunizing not LTA but *S. aureus*. This can be the reason for the result that the monoclonal antibody shows a lower binding efficiency against LTA than the polyclonal antibody.

Table 1. EC50 and LOD Values of Anti-*S. aureus* Antibodies that were Determined from the Titration Curves of Indirect ELISA

antigen	antibody	EC50 (CFU)	LOD (CFU)
LTA	polyclonal Ab	$4.49 \pm 0.64 \times 10^3$	5.32
LTA	monoclonal Ab	n.d	n.d
MSSA	polyclonal Ab	$9.0 \pm 6.68 \times 10^3$	1.0×10^4
MSSA	monoclonal Ab	$2.7 \pm 0.63 \times 10^3$	n.d
MSSA	recombinant Ab (6DW2)	$6.6 \pm 0.45 \times 10^4$	1.5×10^3
MSSA	recombinant Ab (6DWC)	$4.7 \pm 0.12 \times 10^4$	1.1×10^3
MRSA	polyclonal Ab	$7.2 \pm 7.91 \times 10^3$	7.5×10^3
MRSA	monoclonal Ab	$2.0 \pm 2.64 \times 10^3$	5.4×10^1
MRSA	recombinant Ab (6DW2)	$2.3 \pm 0.09 \times 10^4$	3.6×10^2
MRSA	recombinant Ab (6DWC)	$2.1 \pm 0.17 \times 10^4$	n.d.

2.4. Binding Efficiency of the Commercial Antibody to *S. aureus*. Next, we confirmed the binding efficiency of the two commercial antibodies against *S. aureus*. We cultured MRSA and MSSA, then seeded the series-diluted cells on each well of a plate, and performed indirect ELISA (Figure 4). The titers increased with an antigen-concentration-dependent manner, and EC50 and LOD of the two antibodies against MRSA and MSSA were calculated (Table 1). The monoclonal antibody showed a broader detection range than the polyclonal antibody against both pathogens. Moreover, EC50 and LOD of the monoclonal antibody were higher than those of the polyclonal antibody, suggesting a relatively better usefulness of the monoclonal antibody than the polyclonal antibody for *S. aureus* detection. In particular, the LOD of the monoclonal antibody against MSSA and MRSA was under 100 CFU order, indicating high sensitivity.

2.5. Binding Efficiency of Recombinant Antibodies to *S. aureus*. We confirmed the antigen-binding efficiency of the newly generated three recombinant antibodies. First, we seeded 10^7 CFU of MRSA or MSSA on each well of a plate. As a control, we added PBS instead of *S. aureus*. After blocking, we added each recombinant antibody, followed by the HRP-conjugated anti-human Fc antibody, which binds human Fc that is produced from HEK cells (Figure 5A). As a result, all of the three antibodies showed binding efficiency to MRSA and MSSA, and among them, 6DW2 and 6DWC showed higher signals to the one of 6DWI (Figure 5B). Therefore, we move forward to the next step using 6DW2 and 6DWC. We seeded 10^1−10^7 CFU of series-diluted MRSA or MSSA on each well of a plate and performed indirect ELISA. As a result, the signals increased the antigen-concentration-dependent manner (Figure 5C−F), and EC50 and LOD of each antibody were calculated as presented in Table 1.

Malhotra-Kumar et al. revealed that the diagnostic criterion of MRSA is 10^3 CFU/mL.[5] Although the strain is slightly different, the diagnostic criterion of MRSA pneumonia is 10^4 CFU/mL.[30] Therefore, although the antigen-binding properties of recombinant antibodies were relatively higher than those of commercial antibodies, the LOD values of recombinant antibodies were high enough to detect MRSA and MSSA in clinical samples. It is worth mentioning that the information about the commercial antibodies, including their DNA sequences and production methods, has not been revealed. On the other hand, the sequence information of the

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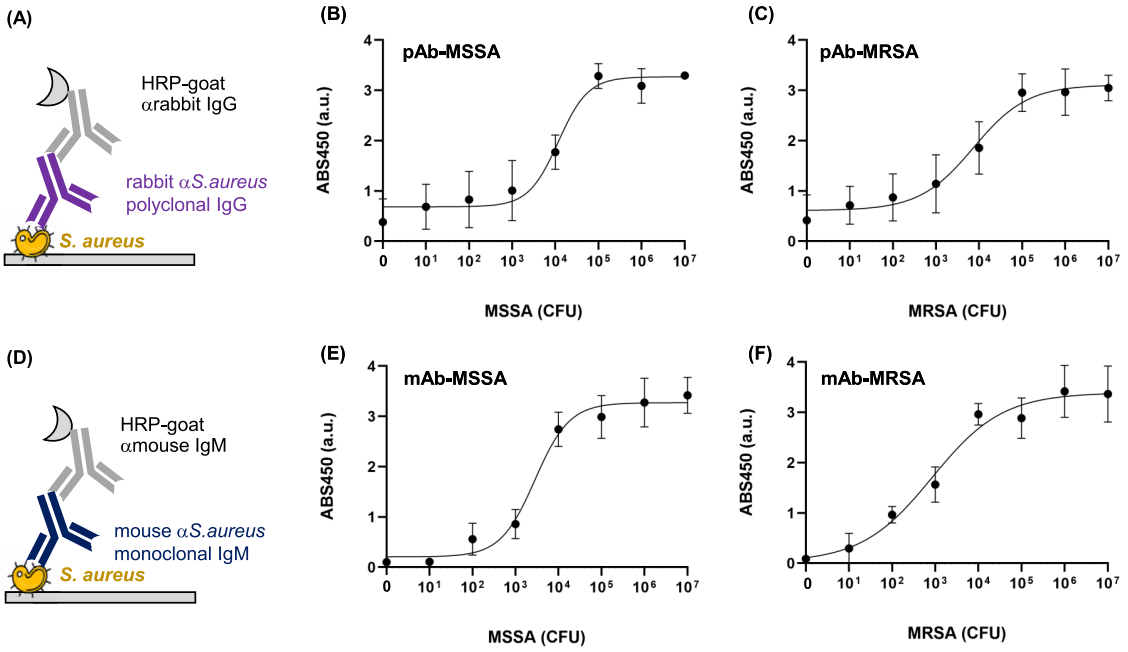

Figure 4. *S. aureus*-binding efficiency of commercial antibodies. (A) Schematic representation of the indirect ELISA for confirming the dose-dependent *S. aureus*-binding efficiency of the polyclonal antibody. (B) ELISA signal of the polyclonal antibody with various concentrations of MSSA. (C) ELISA signal of the polyclonal antibody with various concentrations of MRSA. (D) Schematic representation of the indirect ELISA for confirming the dose-dependent *S. aureus*-binding efficiency of the monoclonal antibody. (E) ELISA signal of the monoclonal antibody with various concentrations of MSSA. (F) ELISA signal of the monoclonal antibody with various concentrations of MRSA. Error bars represent ±1 SD (n = 3).

recombinant antibodies described herein (Table 2) can be widely used for the future development of various modifications, including fluorescent probes or peptide tagging, and for further DNA cloning for making mutants for improving their properties. In addition, detailed methods demonstrated in this study, including mammalian cell-based production of antibodies and confirmation of their binding efficiencies, would be useful for obtaining in-house monoclonal anti-*S. aureus* antibodies with low cost, high yield, and high purity and for applying them for detecting *S. aureus*.

2.6. Sandwich ELISA for Detecting *S. aureus*. We performed not only indirect ELISA but also sandwich ELISA to compare those responses and find the most appropriate detecting system. The important step for sandwich ELISA is selecting the best pair of capturing and detecting antibodies. Therefore, at first, we screened the pairs from the combination of polyclonal, monoclonal, and three kinds of recombinant antibodies. We seeded each antibody to the plate and blocked the empty space of the plate. Afterward, we added 10^1−10^7 CFU of MRSA or MSSA and then washed the plate. Subsequently, we added a capturing antibody, followed by the HRP-conjugated antibody, which binds to the capturing antibody (Figure 6A). As a result, almost all of the pairs, except the polyclonal antibody used as a detecting antibody, showed an antigen-dependent S/B ratio (Figure 6B,C). Among them, the pairs of 6DWC-monoclonal antibody and polyclonal antibody−monoclonal antibody for MSSA, and 6DW2−monoclonal antibody and polyclonal antibody−monoclonal antibody for MRSA showed a higher S/B ratio than others (with the order of the capturing antibody−detecting antibody). Therefore, we moved to the next step using these selected pairs for obtaining titration curves.

We seeded several concentrations of MSSA or MRSA on the plate and performed sandwich ELISA. As a result, the signals were increased with an antigen-concentration-dependent manner, and EC50 and LOD values were calculated (Figure 7, Table 3). Although the LOD values of sandwich ELISA systems were higher than those of indirect ELISA, the sandwich ELISA system can also be used for the practical system because the LOD covers the diagnostic criteria of the concentration of pathogens. As the ELISA method described herein needs below 24 h as an assay time, the total procedure does not require over 2 days even if preincubation for several hours is performed prior to the ELISA method to increase the concentration of pathogens. This merit regarding the rapid procedure time is compared to the traditional methods, including the PCR-based method, which has low selectivity even though the LOD is 10^3 CFU/mL.[5] Boujday et al. developed an immunosensor for detecting MSSA that uses gold-coated sensor chips and presented 10^5 CFU/mL as its detectable concentration.[31] There is a possibility that if the antibody developed in this study was immobilized on the gold-coated sensor chip, the sensitivity can be increased, and the performance of optical sensing can be improved. The ELISA-based method offers a similar or better detectable range and LOD than the other sensing material-based MSSA detecting methods in the use of Ab-quantum dot, (GO/PDMS)/paper, and Ab/NH2-MIL-53.[32−34]

3. CONCLUSIONS

In this study, we produced recombinant anti-*S. aureus* antibodies using mammalian HEK293F cells with high yield and purity. We performed indirect ELISA to confirm the binding efficiency of each antibody to MSSA and MRSA, resulting in very high sensitivity with the order of 10^2 CFU. When we performed sandwich ELISA in the use of recombinant antibodies with commercial antibodies, the selected pairs showed LOD values in the order of 10^4 CFU,

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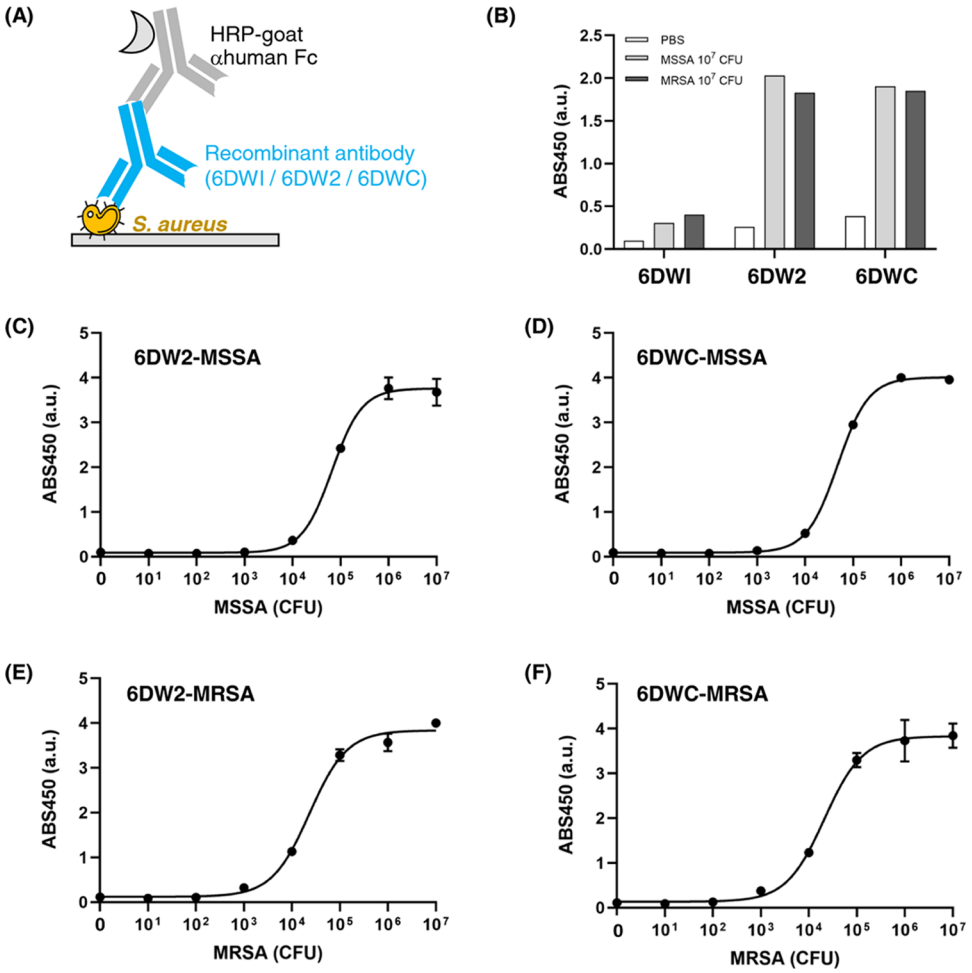

Figure 5. *S. aureus*-binding efficiency of recombinant antibodies. (A) Schematic representation of the indirect ELISA for confirming the dose-dependent *S. aureus*-binding efficiency of recombinant antibodies. (B) ELISA signals of 6DWI, 6DW2, and 6DWc with 10^7 CFU of MSSA or MRSA. (C−F) ELISA signals of 6DW2 or 6DWC with various concentrations of MSSA or MRSA. Error bars represent ±1 SD (n = 3).

which is relatively higher than the value from indirect ELISA but low enough to cover the diagnostic standard of the concentration of pathogens.

The method for producing these antibodies that showed high *S. aureus*-binding efficiency and sensitivity can be used for generating the recombinant anti-*S. aureus* antibody to use it for practical pathogen detection. The procedure to produce a recombinant antibody described herein needs 10 days, which is comparable to the generation of a monoclonal antibody, which needs at least 6 months for mouse immunization followed by hybridoma cell culture or at least 3 months for phage display followed by biopanning. Once a recombinant antibody is produced on a large scale, it can be stored at −20 or −80 °C and then used for each ELISA analysis. It is obvious that the traditional culture and colony-counting method is inadequate for on-the-spot detection because it takes 2−3 days, including the preincubation time to amplify pathogens for obtaining sufficient pathogens. On the other hand, the whole procedure for indirect ELISA using a pre-made antibody described herein needs ∼20 h, which is less time-consuming than the traditional method. Moreover, in the case of sandwich ELISA, the 96-well plate can be precoated to immobilize the capture antibody that provides the assay time down to 4−5 h, which is distinguishable from the conventional detecting methods as well as indirect ELISA when it is developed as a ready-to-use ELISA kit.

The ELISA method with the newly developed recombinant antibody does not require any amplification and can directly detect *S. aureus* with the low limit of detection of 10^2−10^3 CFU order. Moreover, this method has higher selectivity and sensitivity comparable to the conventional methods to detect *S. aureus*. The rapid and sensitive *S. aureus* detecting method using a newly developed recombinant antibody described in this study can be used for efficient detection of *S. aureus* with a wide range of applications including medical and pharmaceutical diagnosis, antibiotic discovery, and food safety.

4. METHODS

4.1. Materials. Herculase II Fusion DNA polymerase was obtained from Agilent (Seoul, Korea). Oligonucleotides were obtained from Bionics (Seoul, Korea). The plasmid miniprep kit was obtained from GeneAll Biotechnology (Seoul, Korea). Protein A beads were obtained from GE healthcare (Piscataway, NJ). A disposable gravity column was obtained from Bio-Rad (Daejeon, Korea). Ultrafiltration devices were obtained from Millipore (centrifugal filter tube Ultra-4, MWCO 3k; Seoul, Korea). The 96-well Maxi-binding ELISA plate was obtained from SPL (Seoul, Korea). Freestyle 293 medium was obtained from Gibco (Waltham, MA). PEI was obtained from

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Table 2. Nucleotide Sequences of Variable Domains of Recombinant Antibodies

antibody	VH	VL
6DWI	caggtccagctgcagcactctggcggcggcttgaacaacctggcggcagcctgagaatcagctgtgccgcttccggcttcaccttcaacaccaacgacatgagc tgggtccgacaggccccttggaaaaggactgcagtgggtgtccacatatcggcatcgacacacacactacgccgattctgtgcgggcagattcaccgttccaga- gacaccagcaagaacatggtgtacctgcagatgaactccctgcgtgaagatacagccctgtactactgcgtgaagaacagcggcatctacag- tttgggggccaggcacactcgtgacagtg	gacatccagatgacacagagccccgccacactgagtgtctccagcggaaacagtgacctgagctgcagagcctctcagagcgtgcggacaaacgtggcc tggtacagacacaagccggacagagccctatgatcctggtgctggcgcctctacaagagcctcctggtgccctgccagatttctggctctggc tacggcaccgagttcaccctgacaatcacaagcctgcagagcgagacttcgccgtgtactactgcctgcagtacaacacctggcc tcggacatttggccaggcaccaaggtggaagtg
6DW2	gaagtgcagctggtgcagtctggcgccgaagtgaaaaaacctggcgcctccgtgaaggtgtcctgtgaagcctctggctacaccctgaccagctacgacatcaac tgggtccgacaggccacaggacagggactgaatggatgggctggatgaacgccaacagcggctacgcccagaaattccagggcagagtgaccc tgacaggcgacacctctatcagcaccgcctacatggaactgagcagcctgagaagcgaggacacgccgtgtactactgtgcgggggagccc tggcagatacttcgatctttgggggcagaggcaccctcgtgacagtg	gatatccagctgacacagagccccgatagcctggccgtgtctctgggagaaagagccaccatcaactgcaaggagcagccagagcatc ttccggaccagcgggacaagaacctgctgaatctggtatcagcagcggccttggccaacctccctagactgctgatttcac tgggccagcaccagaagctcggcgtgcagatagattttccggcagcggcttcggcaccgacttcaccctgacaatcatccctgcaggccgag- gacgtggccatctactactgccagcagtacttcagccctcctttacacctttggccagggcaccaagctggaaatc
6DWC	ggaggtgcagctggtggaatctggcggcggacttgttcaacctggcggctctctgagactgagctgttctgccagcggcttcagcttcaacagcttctggatgcac tgggtccgacaggtgccaggcaaagggactctggtggatcagtctgttagaggccggttcatcatcagccgg- gacaacgccaagaacaccctgtacctggaaatgaacaactgagaggcgaggacacgccgtgtactattgtgctagaggcgacggcggcctggatgattggggga- cagggaacactggtcaccgtg	gacatcgtgatgacacagagccccgacagcctgtctgtgtctctgggcgagagagtgaccatcaactgtagagccagcgagaccatctctggatggctggcc tggtatcagcagaagcctgccgaggctcctaagctgctgatctacaagggcggctgtgcccagcgatttttc tggcagcggaagcggcaccgagttcaccctgaccatcatctgcagcctgcagagctccgacgattgccagtactactgccagcagtacaagagctacagcttcaac ttcggccagggcaccaagctggaaatc

Polysciences (Warrington, PA). LTA was obtained from Sigma (Seoul, Korea). Goat anti-mouse IgG HRP was obtained from Pierce (Seoul, Korea). Mouse anti-*S. aureus* monoclonal IgM and rabbit anti-*S. aureus* polyclonal IgG were obtained from Thermo. HRP-conjugated goat anti-rabbit IgG (H+L) and HRP-conjugated goat anti-mouse IgM were obtained from Invitrogen (Waltham, MA). Other chemicals and reagents, unless otherwise indicated, were from Sigma.

4.2. Gene Construction of the Recombinant Anti-*S. aureus* Antibody. VH or VL of anti-*S. aureus* antibody expressing genes, 6DWI, 6DW2, and 6DWC, were codon-optimized and amplified by polymerase chain reaction (PCR) using primers (Table 4), Herculase II Fusion DNA polymerase, and synthesized DNA (Table 2) as a template. pcDNA3.1(−) was amplified by PCR using primers (Table 4). Each insert PCR product and vector PCR product was ligated using an infusion enzyme, resulting in pcDNA3.1-(6DWI-VH), pcDNA3.1(6DW2-VH), pcDNA3.1(6DWC-VH), pcDNA3.1(6DWI-VL), pcDNA3.1(6DW2-VL), and pcDNA3.1(6DWC-VL). The obtained plasmids were prepared using the plasmid miniprep system, and the entire coding region sequences were confirmed by sequencing. Afterward, large-scale plasmid preparation using the plasmid maxiprep system was performed.

4.3. Cell Culture and Production of Recombinant Antibody. HEK293FT cells were maintained in 50 mL Freestyle 293 growth medium in a 250 mL flask in an atmosphere of 8.0% CO_2 at 37 °C. After a suspension cell culture, cells in the supernatant (3×10^5−3×10^6 cells/mL) were obtained, and the subcultured cells were transferred to the 200 mL media with the density of 1×10^6 cells/mL. Then, 1.25 μg/mL HC and LC genes of the antibody were successively cotransfected to HEK293F cells using 7.5 μg/mL PEI according to the manufacturer's protocol. After 7 days of incubation with 120 rpm at 8.0% CO_2, 37 °C, the supernatant was transferred to a 50 mL tube and the cells were collected by centrifugation (1000 rpm, 5 min, 4 °C).

4.4. Purification of the Recombinant Antibody. Recombinant antibodies obtained from each cell supernatant were purified using PA agarose beads as follows: 10 μL of the resin slurry was added to 1 mL of the cell supernatant and then incubated at RT for 1 h. After washing using 500 μL of PBS three times, the antibody was eluted using 100 μL of 0.1 M glycine (pH 2.5) and the eluted sample was immediately adjusted to neutral by adding 10 μL of PBS. The antibody concentration was determined by measuring the absorbance at 280 nm using nanodrops.

4.5. *S. aureus* Culture. The cells of two *S. aureus* strains, MSSA (*S. aureus* WKZ-1, NR-28984) and MRSA (*S. aureus* WKZ-2, NR-28985),[35] were cultured by growing cultures overnight to log phase at 37 °C at 225 rpm. The cultures at OD600 = 0.6 were washed three times with PBS and diluted with the same buffer.

4.6. Confirmation of the Antigen-Binding Activity. The antigen-binding activities of recombinant and commercial antibodies were tested via indirect ELISA as follows: 100 μL of series-diluted LTA or *S. aureus* was immobilized on a 96-well plate for 37 h at 2 °C. After that, the well was filled with 200 μL of TBST that contains 3% BSA for 1 h at 37 °C and then washed three times with TBST. Subsequently, 500 ng/mL of the antibody in 100 μL of PBS with 0.1% BSA was added and incubated for 1 h at 25 °C. After washing three times with 200 μL of TBST, the bound protein was probed with 5000-fold

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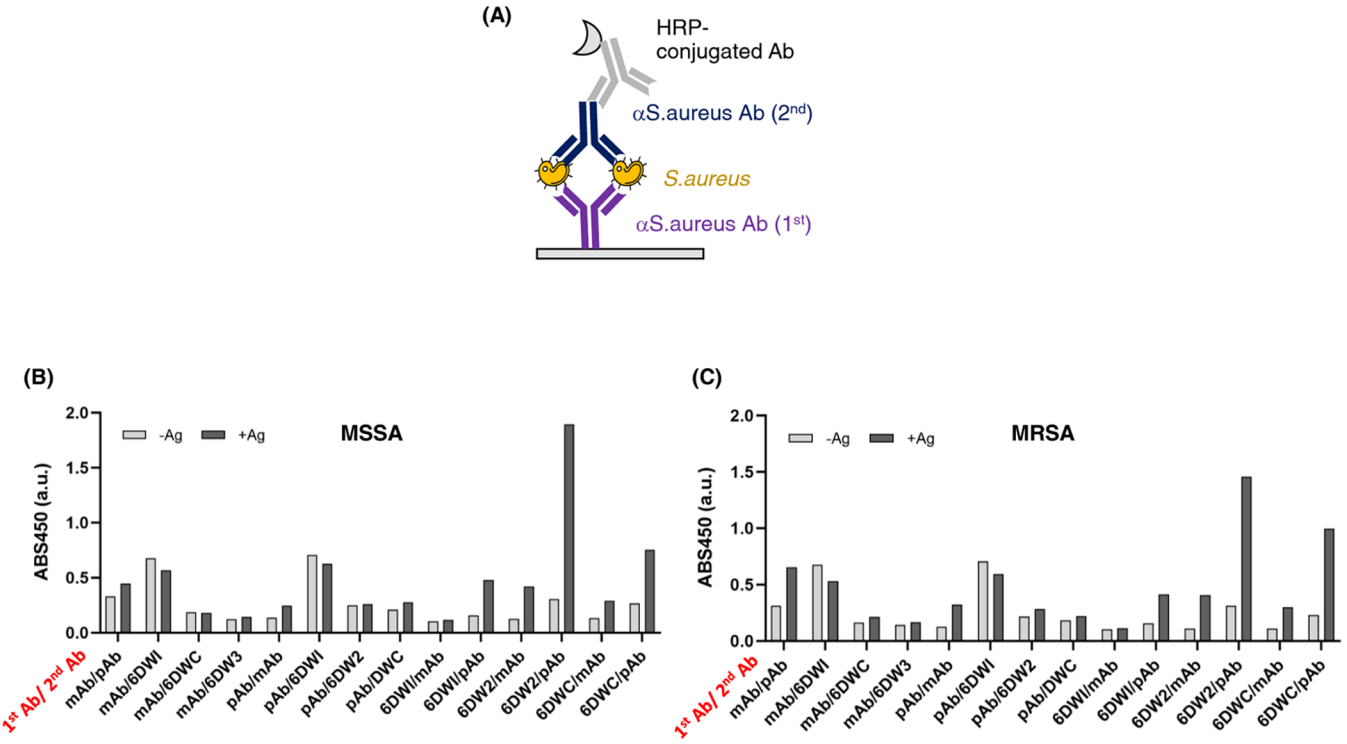

Figure 6. (A) Schematic representation of sandwich ELISA. (B) ELISA signals of each pair with 10^8 CFU of MSSA. (C) ELISA signals of each pair with 10^8 CFU of MRSA. PBS was added instead of MSSA or MRSA as a negative control.

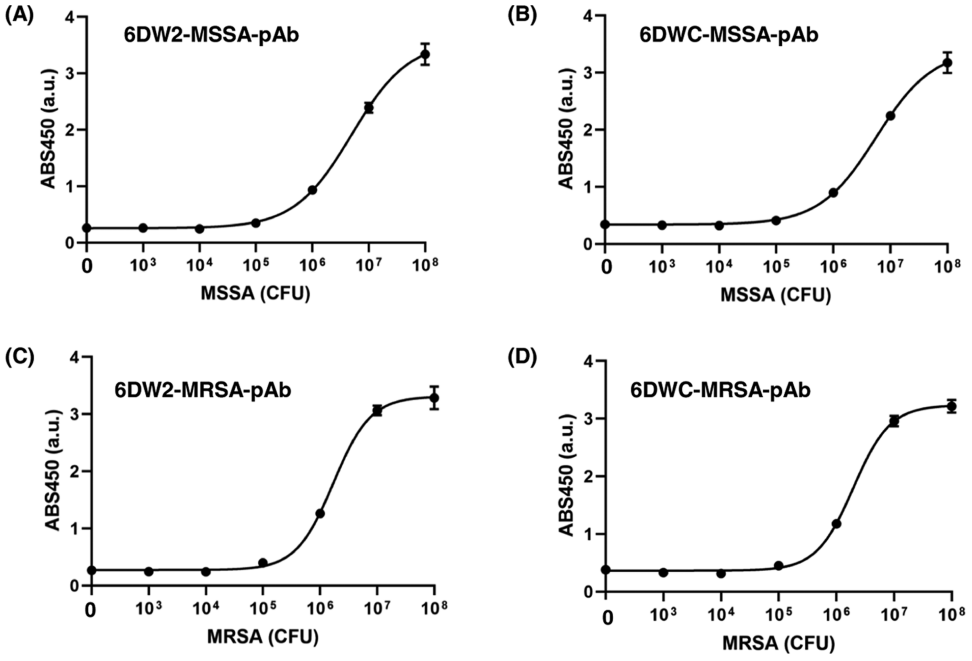

Figure 7. (A) Titration curve for detecting MSSA via sandwich ELISA using 6DW2 as a capture antibody and polyclonal antibody as a detecting antibody. (B) Titration curve for detecting MSSA via sandwich ELISA using 6DWC as a capture antibody and polyclonal antibody as a detecting antibody. (C) Titration curve for detecting MSSA via sandwich ELISA using 6DW2 as a capture antibody and monoclonal antibody as a detecting antibody. (D) Titration curve for detecting MRSA via sandwich ELISA using the 6DWC antibody as a capture antibody and polyclonal antibody as a detecting antibody. Error bars represent ±1 SD (n = 3).

diluted HRP-conjugated goat anti-mouse IgG antibody in PBS with 0.5% BSA for 1 h at 25 °C. The well was washed three times with 200 μL of TBST and developed with 100 μL of the TMB solution. After incubation for 10 min, the reaction was stopped with 50 μL of 1 N H_2SO_4, and the absorbance was read at 450 nm using a microplate reader. Dose−response curves were constructed by fitting the intensities at a maximum emission wavelength of spectra using the Graphpad Prism. The EC50 value was calculated from the curve fitting to a four-parameter logistic equation. The LOD value was calculated

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Table 3. EC50 and LOD Values of Anti-*S. aureus* Antibodies that were Determined from the Titration Curves of Sandwich ELISA

antigen	capturing antibody	detecting antibody	EC50 (CFU)	LOD (CFU)
MSSA	recombinant Ab (6DW2)	polyclonal Ab	$5.2 \pm 1.74 \times 10^6$	6.2×10^4
MSSA	recombinant Ab (6DWC)	polyclonal Ab	$1.7 \pm 0.27 \times 10^6$	9.3×10^4
MRSA	recombinant Ab (6DW2)	polyclonal Ab	$5.8 \pm 1.91 \times 10^6$	4.8×10^4
MRSA	recombinant Ab (6DWC)	polyclonal Ab	$1.9 \pm 0.10 \times 10^6$	n.d

Table 4. Primers Used in This Study

		insert PCR (5′−3′)		vector PCR (5′−3′)	
6DWI	H	IL_6DWI VHfuF	6DWI_fcfuR	IL_6DWI VHfuR	6DWI_fcfuF
		tggtcaccaatagcgacatccagatgacacagag	cggccactgttctcttcacttccaccttggtgcc	tgtgtcatctggatgtcgc tattggtgaccaggg	accaaggtggaagtgaagagaacagtggccgctc
	L	IL_6DWI VLfuF	6DWI_CLfuR	IL_6DWI VLfuR	6DWI_CLfuF
		cctggtcaccaattctcaggtccagctgcagcac	ttgtagaggcgctagacac tgtcacgagtgtgcc	gctgcagctggacc tgagaattggtgaccaggg	acactcgtgacagtgtctagcgcctctacaaagg
6DW2	H	IL2_6DW2VHfuF	6DW2_FcfuR	IL2_6DW2VH_fuR	6DW2_FcfuF
		ctggtcaccaattctgaagtgcagctggtgcag	gtagaggcgctagacac tgtcacgagggtgcctc	gcaccagctgcac ttcagaattggtgaccaggg	accctcgtgacagtgtctagcgcctctacaaagg
	L	IL_6DW2VLfuF	6DW2_CLfuR	IL_6DW2VLfuR	6DW2_CLfuF
		cctggtcaccaatagcgacatcgtgatgacacag	ggccactgttctcttgatttccaccttggtgcc	gtgtcatcacgatgtcgc tattggtgaccaggg	accaaggtggaaatcaagagaacagtggccgctc
6DWC	H	IL_6DWC VHfuF	6DWC_FcfuR	IL_6DWC VH_fuR	6DWC_FcfuF
		tggtcaccaattctgaggtgcagctggtggaatc	ttgtagaggcgc tagacacggtgaccagtgttcc	ccaccagctgcacc tcagaattggtgaccagggc	acactggtcaccgtgtctagcgcctctacaaagg
	L	IL_6DWC VLfuF	6DWC_VLfuR	IL_6DWC VL_fuR	6DWC_CLfuF
		tggtcaccaatagcgatatccagctgacacagag	cggccactgttctcttgatttccagcttggtgcc	gtgtcagctggatatcgc tattggtgaccagggc	accaagctggaaatcaagagaacagtggccgctc

based on the equation Y = bottom + (top − bottom)/(1 + 10((log EC50 − X) × HillSlope)) using GraphPad Prism version 8.0.

4.7. Sandwich ELISA. Recombinant or commercial antibody (300 ng) was immobilized on the 96-well plate for 16 h at 4 °C, and the well was filled with 350 μL of PBS buffer that contains 3% BSA for 1 h at 37 °C and washed three times with 350 μL of PBS buffer that contains 0.1% BSA. Subsequently, 100 μL of *S. aureus* was added and incubated for 1 h at 25 °C. After washing three times with 350 μL of 0.1% BSA in PBS, the bound protein was probed with 300 ng of recombinant or commercial antibody for 1 h at 25 °C. After washing three times with 350 μL of 0.1% BSA in PBS, HRP-conjugated rabbit anti-human Fc antibody for the recombinant antibody, anti-mouse IgM for the monoclonal antibody, or anti-rabbit IgG for the polyclonal antibody was added in 0.1% BSA in PBS for 1 h at 25 °C. The well was washed three times with 350 μL of 0.1% BSA in PBS and developed with 50 μL of TMB solution. After incubation for 15 min, the reaction was stopped with 25 μL of 1 N H_2SO_4, and the absorbance was read at 450 nm using a microplate reader Model 680 (Bio-Rad). Dose−response curves were constructed by fitting the intensities at the maximum emission wavelength of spectra using the Graphpad Prism. The EC50 value was calculated from the curve fitting to a four-parameter logistic equation. The LOD value was calculated based on the equation Y = bottom + (top − bottom)/(1 + 10((log EC50 − X) × HillSlope)) using GraphPad Prism version 8.0.

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Notes

The authors declare no competing financial interest.

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