Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

One-step immunoassay for microbial detection using isolated antibodies from un-immunized horse serum

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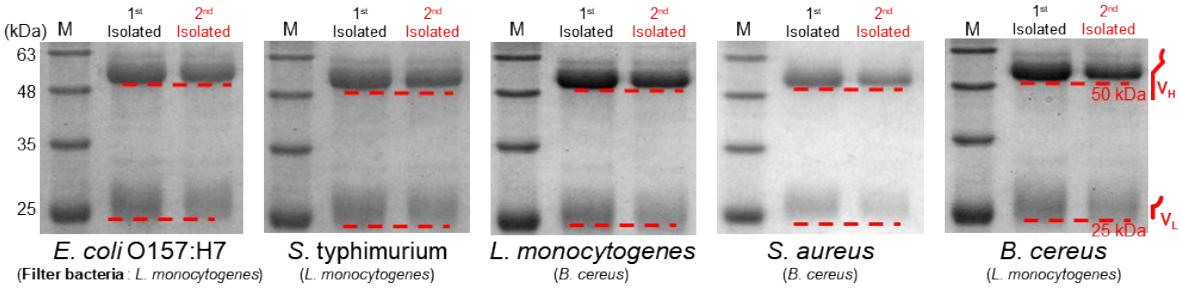
Supplementary Fig. S1. SDS-PAGE of antibodies through stepwise filtration for each target strain of *E. coli* O157:H7, *S. typhimurium, L. monocytogenes*, *S. aureus*, and *B. cereus*.

(a)



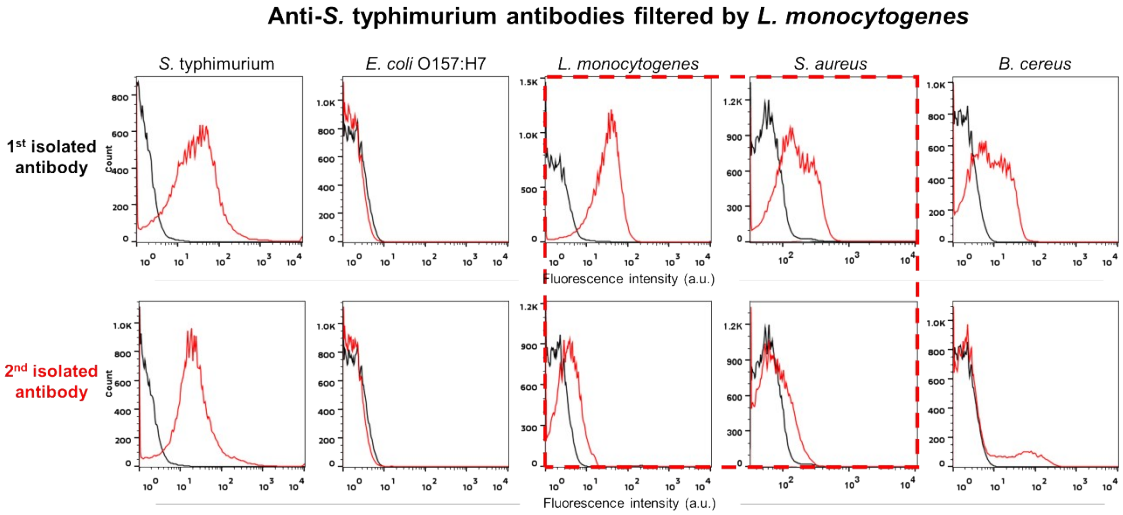
Supplementary Table S1. Binding properties of the antibodies against bacteria isolated from horse serum. The binding activity of the isolated antibodies to each strain was indexed by calculating the proportion of the binding activity to the target bacteria as 100 %. In this work, "+++++" represented the binding activity to the target bacteria, and each "+" represented a difference of 20 % from the activity of the target bacteria (100%).

("+",20% of the signal from the target bacteria)

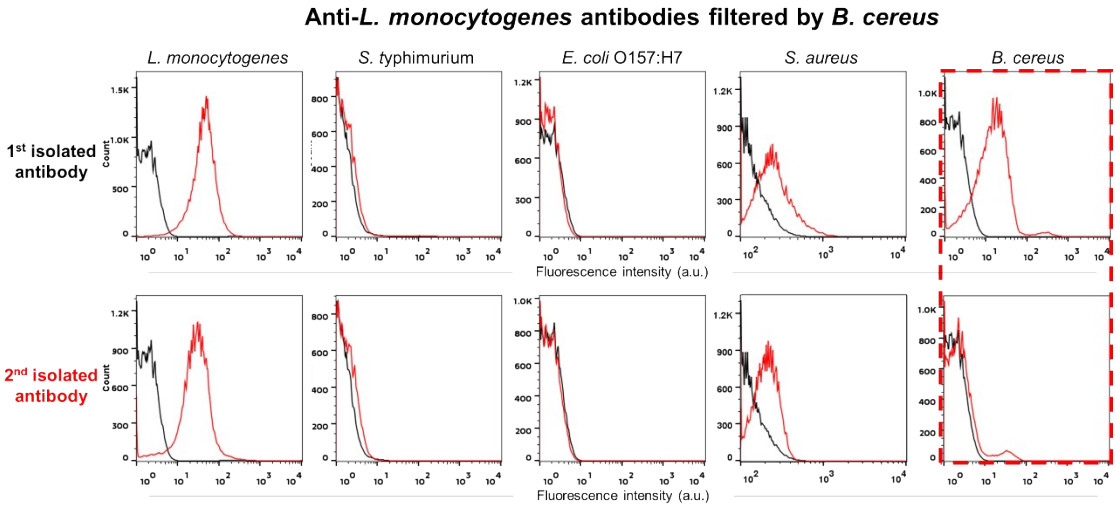
Target bacteria (Filter bacteria)		**Normalized binding affinity** ("+",20% of the signal from the target bacteria)				
		E.coli O157:H7	*S.* typhimurium	*L. monocytogenes*	*S. aureus*	*B. cereus*
***E. coli* O157:H7** (*L. monocytogenes*)	1st isolated	+++++	-	++++	+++	-
	2nd isolated	+++++	-	-	-	-
S. typhimurium (*L. monocytogenes*)	1st isolated	-	+++++	++++	+	-
	2nd isolated	-	+++++	-	-	-
L. monocytogenes (*B. cereus*)	1st isolated	-	-	+++++	-	+
	2nd isolated	-	-	+++++	-	-
S. aureus (*B. cereus*)	1st isolated	-	-	-	+++++	++++
	2nd isolated	-	-	-	+++++	-
B. cereus (*L. monocytogenes*)	1st isolated	-	-	+++	+	+++++
	2nd isolated	-	-	-	-	+++++

Supplementary Fig. S2. Analysis of cross reactivity of antibodies isolated by stepwise filtration. Flow cytometric analysis of cross reactivity for the isolated antibodies (1st and 2nd-filtered antibodies) against other strains. (a) *S. typhimurium,* (b) *L. monocytogenes*, (c) *S. aureus* and (d) *B. cereus*.

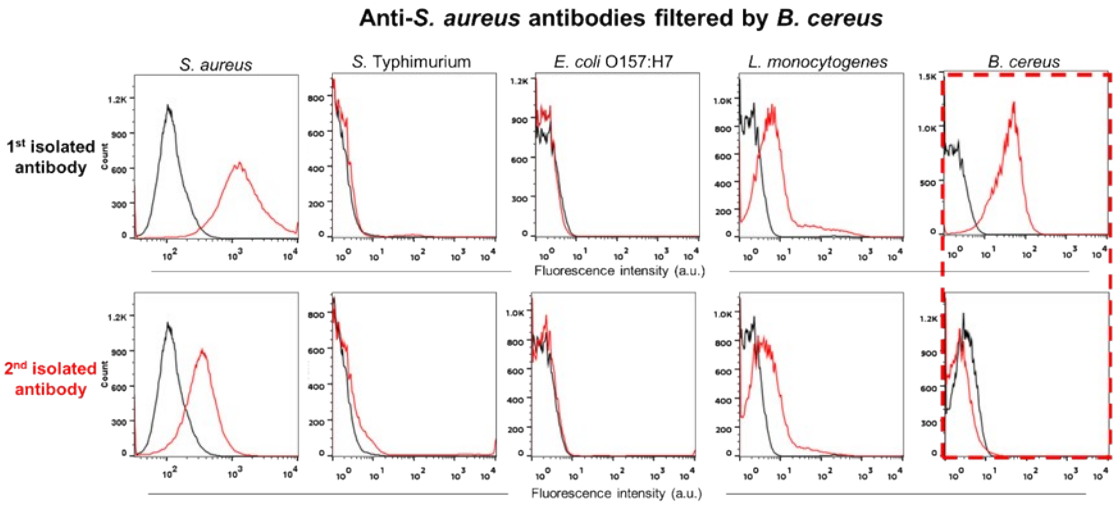
(a)



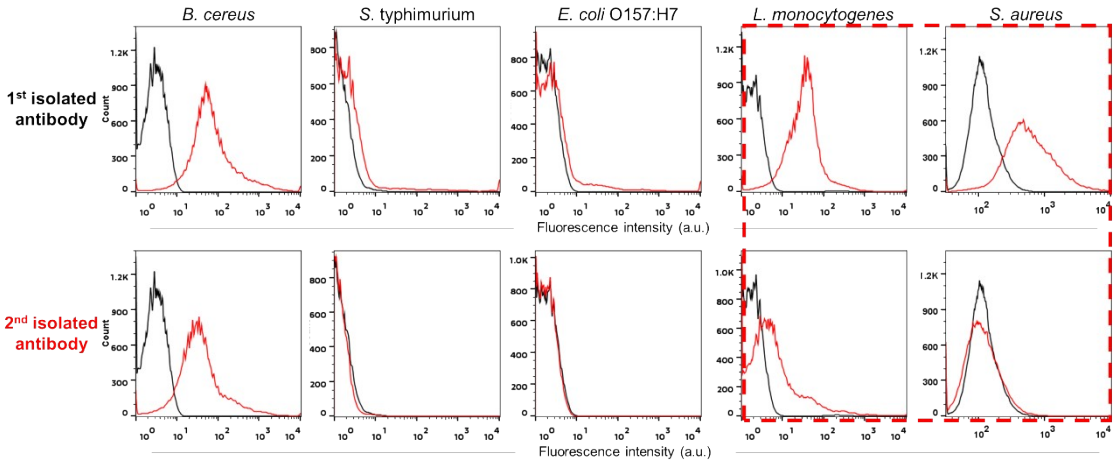
(b)



(c)

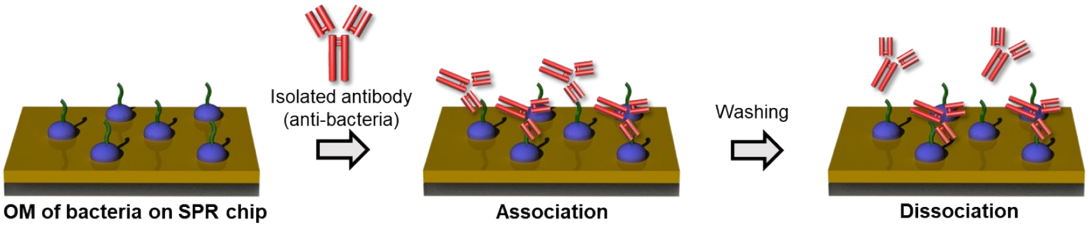

Anti-S. aureus antibodies filtered by B. cereus
S. aureus
S. Typhimurium
E. coli O157:H7
L. monocytogenes
B. cereus
1st isolated antibody
2nd isolated antibody
Count
Fluorescence intensity (a.u.)

(d)

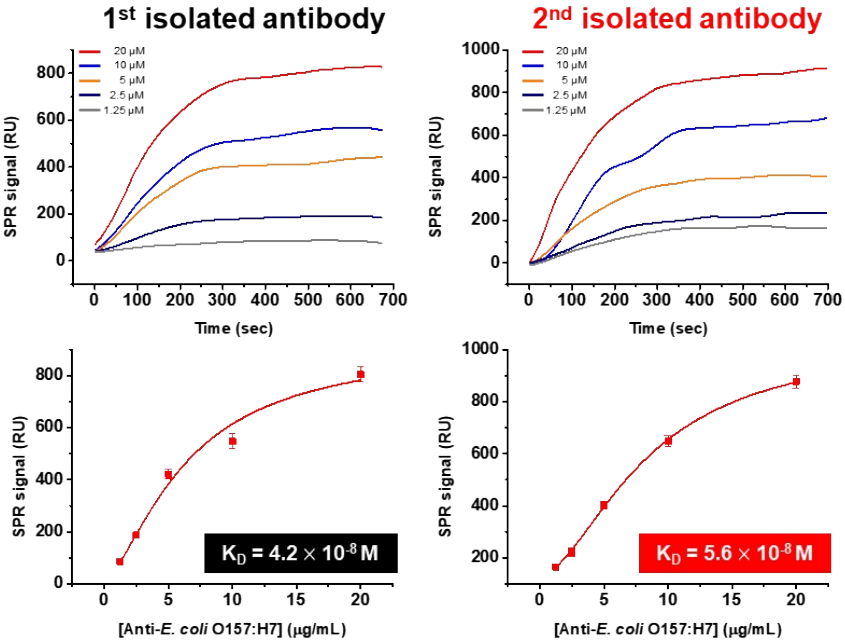

Anti-B. cereus antibodies filtered by L. monocytogenes
B. cereus
S. typhimurium
E. coli O157:H7
L. monocytogenes
S. aureus
1st isolated antibody
2nd isolated antibody
Count
Fluorescence intensity (a.u.)

Supplementary Fig. S3. Estimation of binding constant (K_D) of the isolated antibodies against *E. coli* O157:H7. (a) Schematic view of SPR measurement. The SPR sensor graph and the Hill's plot from the treatment of (b) the 1st antibodies and 2nd antibodies. Analysis results for other strains were (c) *S. typhimurium,* (d) *L. monocytogenes*, (e) *S. aureus*, and (f) *B. cereus*.

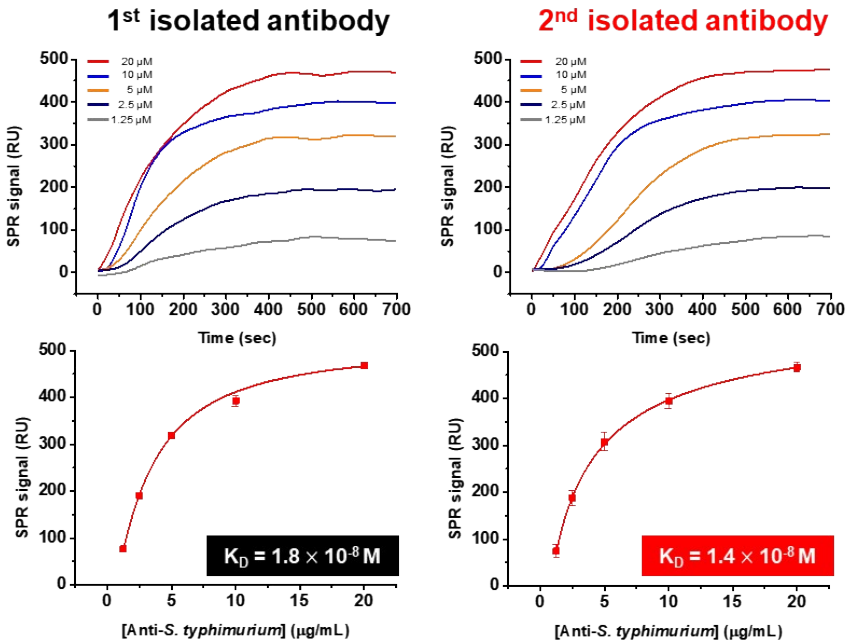
(a)



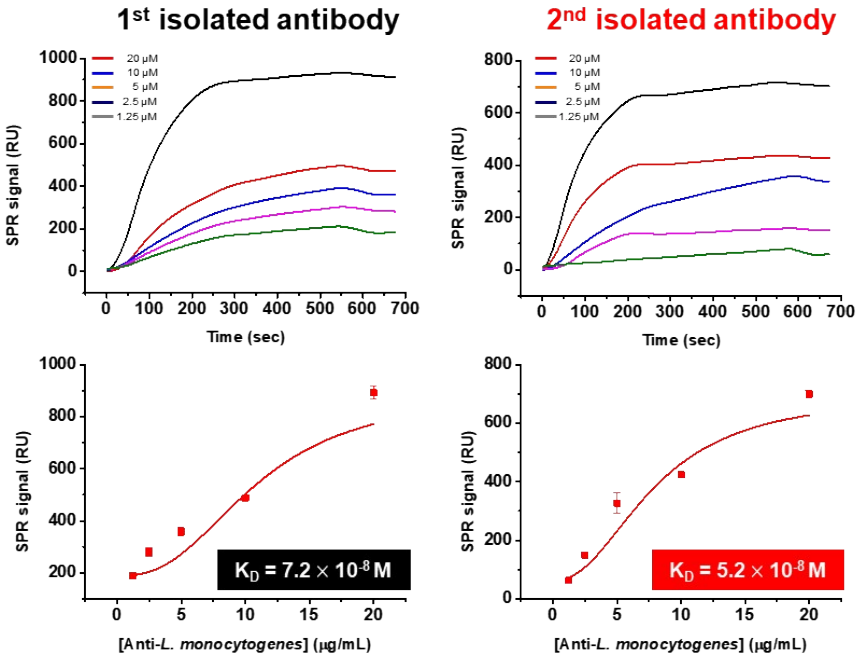
(b) Estimation of binding constant (K_D) of the isolated antibodies against *E. coli* O157:H7



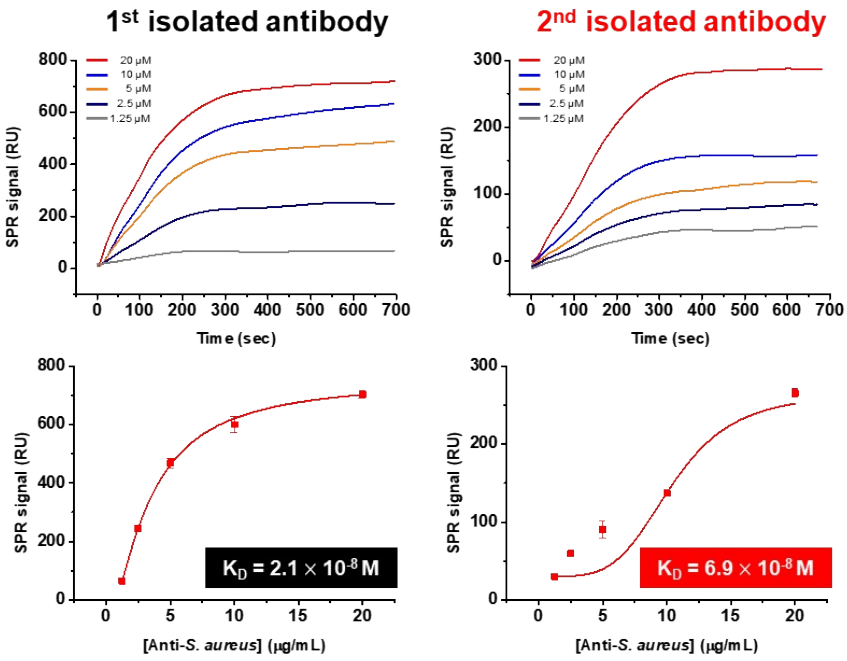
(c) Estimation of binding constant (K_D) of the isolated antibodies against *S. typhimurium.*



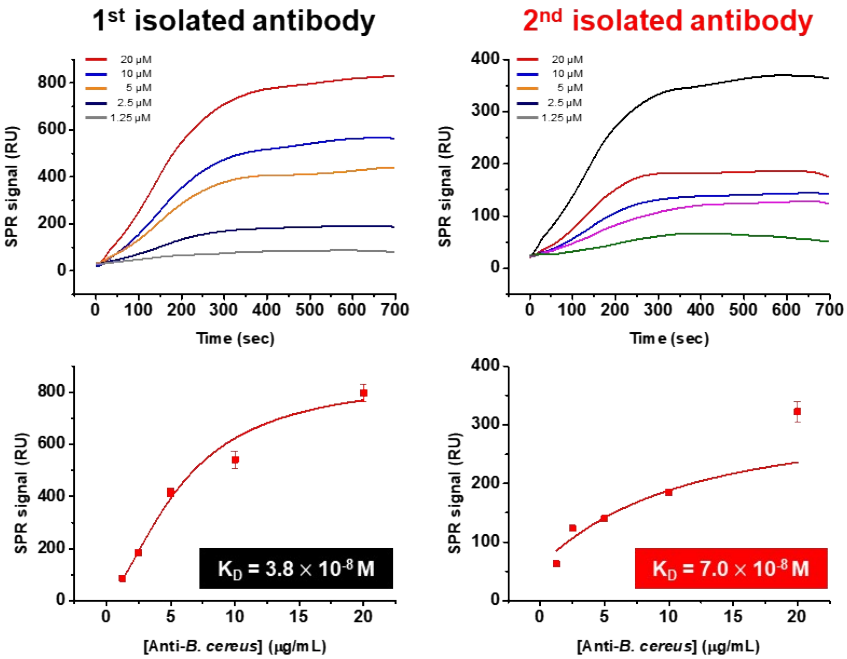
(d) Estimation of binding constant (K_D) of the isolated antibodies against *L. monocytogenes.*



(e) Estimation of binding constant (K_D) of the isolated antibodies against *S. aureus.*

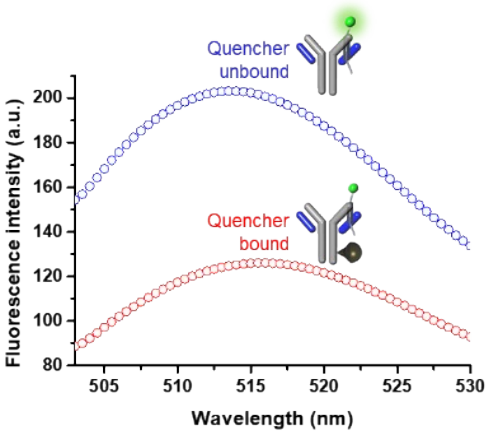


(f) Estimation of binding constant (K_D) of the isolated antibodies against *B. cereus.*



Supplement Fig. S4. Estimation of quenching effect of labeled organic quencher. (a) Fluorescence spectra for the switching peptide bound antibodies with and without organic quencher. The spectrum was measured at the excitation wavelength of 470 nm and emission wavelength range of 500-530 nm. (b) Comparison of fluorescence signal from switching peptide, switching peptide bound antibodies without and with organic quencher (2nd and 3rd signal) and immobilized antibodies bound with switching peptide.

(a)



(b)