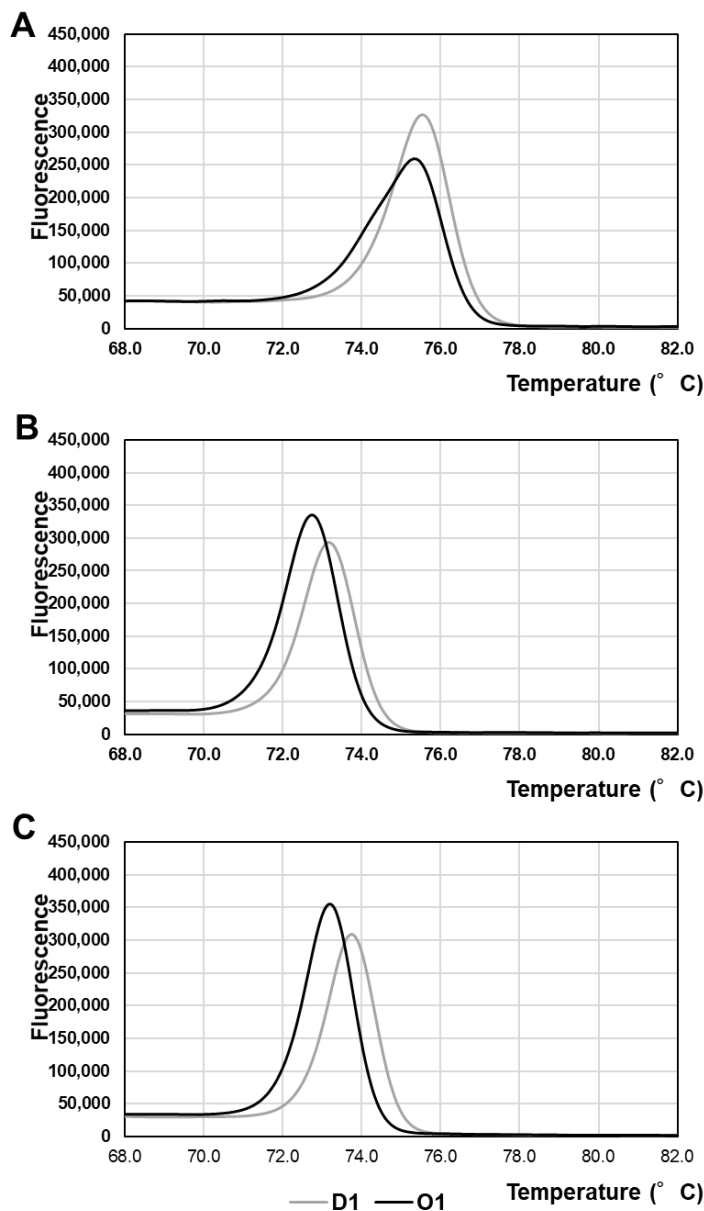**Supplementary Figure 1. Derivative HRM curves for amplicons from cDNA reference standards of SARS-CoV-2 isolate Wuhan-Hu-1 RBD and SARS-CoV-2 RBD variants.**
(A) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second G339D forward" and "Second G339D reverse" to detect the G22578A mutation.
(B) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second L452R forward" and "Second L452R reverse" to detect the T22917G mutation.
(C) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second D796Y forward" and "Second D796Y reverse" to detect the G23948T mutation.

Black dotted lines indicate HRM curves for amplicons from the cDNA reference standard of SARS-CoV-2 isolate Wuhan-Hu-1 RBD. Grey broken lines indicate HRM curves for amplicons from the cDNA reference standard of SARS-CoV-2 Alfa variant RBD. Grey solid lines indicate HRM curves f for amplicons from the cDNA reference standard of SARS-CoV-2 Delta variant RBD. Black solid lines indicate HRM curves for amplicons from the cDNA reference standard of SARS-CoV-2 Omicron variant RBD. HRM analyses were performed three times to obtain the mean value of Tm. A representative curve is shown in this figure.
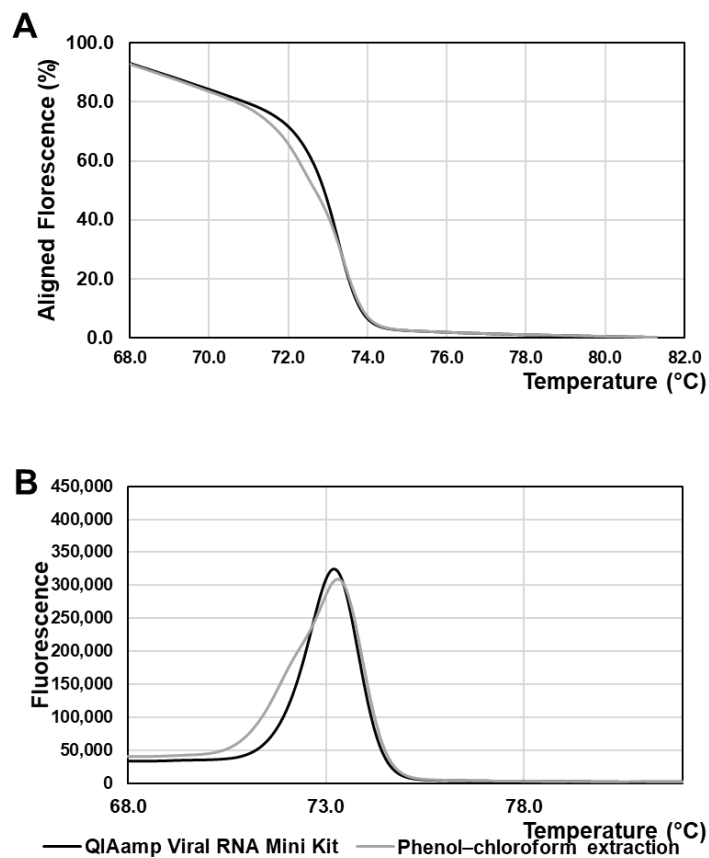
Supplementary Figure 1

Supplementary Figure 2. Derivative HRM curves of amplicons of extracted viral RNA from Delta variant-positive D1 specimens and Omicron variant-positive O1 specimens.

(A) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second G339D forward" and "Second G339D reverse" to detect the G22578A mutation.

(B) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second L452R forward" and "Second L452R reverse" to detect the T22917G mutation.

(C) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer pair "Second D796Y forward" and "Second D796Y reverse" to detect the G23948T mutation.

Grey solid lines indicate HRM curves of amplicons of extracted viral RNA from Delta variant-positive D1 specimens. Black solid lines indicate HRM curves of amplicons of extracted viral RNA from Omicron variant-positive O1 specimens.

Supplementary Figure 2

Supplementary Figure 3. Normalized and derivative HRM curves of amplicons of extracted viral RNA from Omicron variant-positive O2 specimens.

(A) Normalized HRM curves of amplicons from the second amplification by nested PCR with the primer sets "Second D796Y forward" and "Second D796Y reverse" to detect the G23948T mutation.

(B) Derivative HRM curves of amplicons from the second amplification by nested PCR with the primer sets "Second D796Y forward" and "Second D796Y reverse" to detect the G23948T mutation.

Grey solid lines indicate HRM curves of extraction by phenol–chloroform from Omicron variant positive O2 specimens. Black solid lines indicate HRM curves of extraction by QIAamp Viral RNA Mini Kit from Omicron variant positive O2 specimen.