

Figure S1. Construction of pUC57 (positive controls) carrying the consensus sequences of target genes used in melting curve-based multiplex real-time PCR. **(a)** RNA-dependent RNA polymerase (RdRp) and the nucleocapsid phosphoprotein N of SARS-CoV-2; **(b)** Matrix protein 2 of Influenza A virus (FLU-A); **(c)** and the RdRp domain of L protein from Human Respiratory Syncytial Virus (HRSV), and the 5'-UTR polyprotein from Human Rhinovirus B (HRV-B).

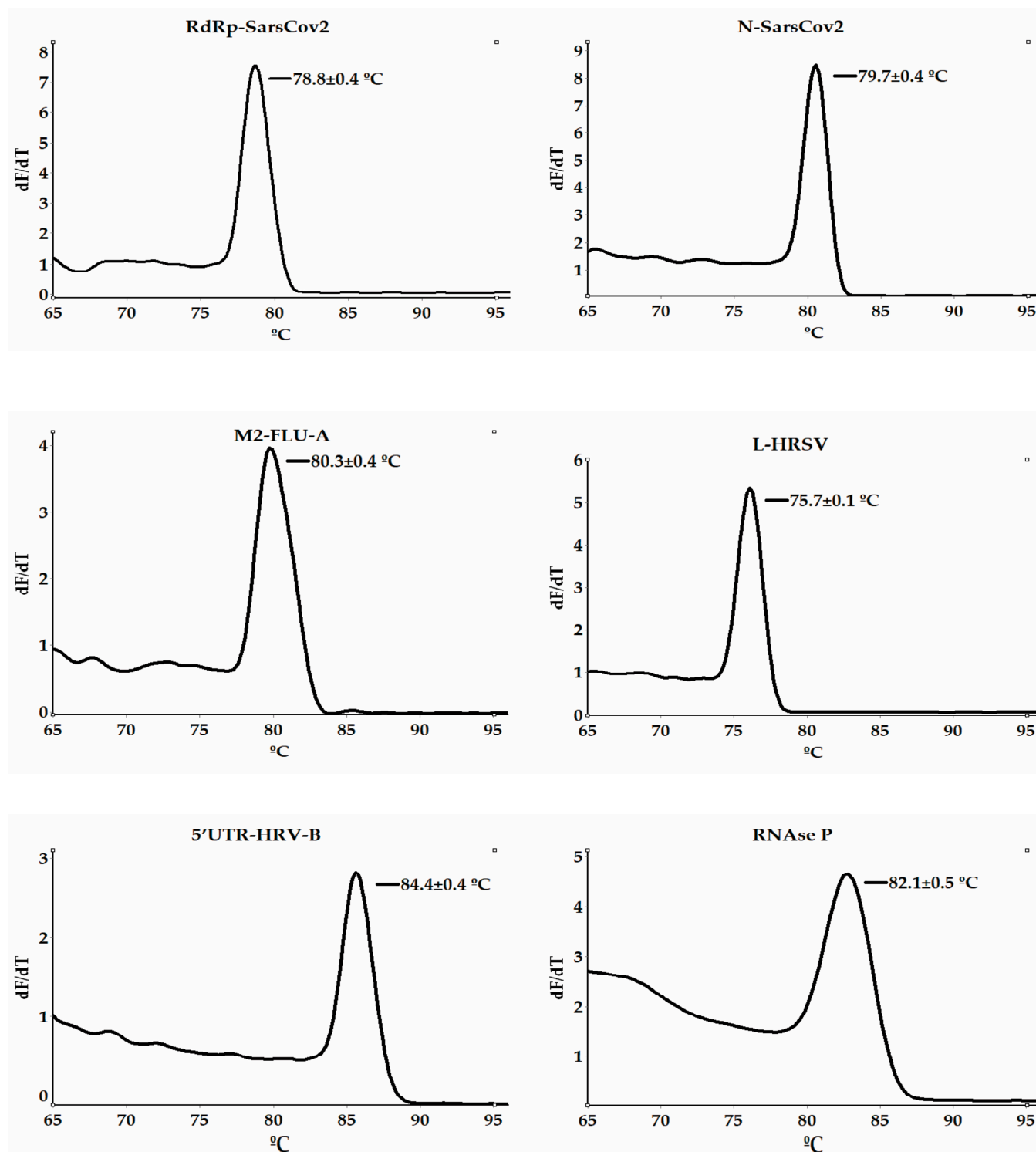


Figure S2. Monoplex real-time PCR for determining melting temperature peaks (T_m) of RNA-dependent RNA polymerase (RdRp) and the nucleocapsid phosphoprotein (N) of SARS-CoV-2, the Matrix protein 2 (M2) of Influenza A virus (FLU-A), the RdRp domain of L protein (L) from Human Respiratory Syncytial Virus (HRSV), the 5'-UTR polyprotein from Human Rhinovirus B (HRV-B) amplicons using positive (plasmid) controls. RNase P amplicon T_m peak was determined using human nasopharyngeal swab samples.

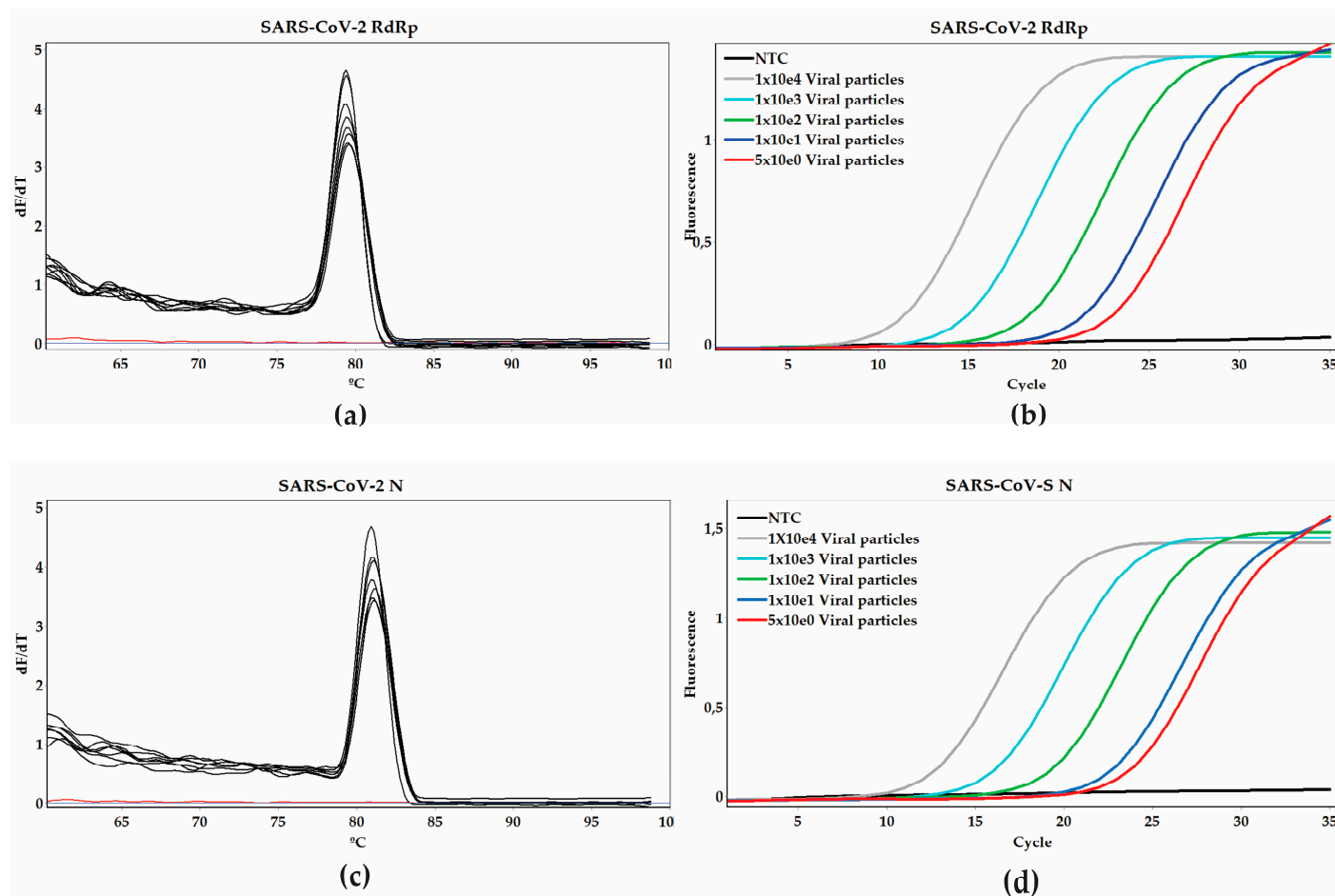


Figure S3. Performance of real-time PCR assay for SARS-CoV-2. Melting temperature peaks (T_m) of (a) RNA-dependent RNA polymerase (RdRp) and (c) nucleocapsid phosphoprotein N of SARS-CoV-2 (c) using SARS-CoV-2/human/BRA/SP02cc/2020 culture in Vero ATCC CCL81 cells. Sensitivity of real-time PCR assays: Amplification pattern of serial dilution corresponding to 10000, 1000, 100, 10, and 5 viral particles. The virus titration was determined by tissue culture infectious dose (TCID₅₀/mL) assay [37]. (b) RNA-dependent RNA polymerase (RdRp) and (d) nucleocapsid phosphoprotein N.