Supporting Information for

One-pot, Ultrasensitive, and Multiplex Detection of SARS-CoV-2 Genes Utilizing Self-Priming Hairpin-Mediated Isothermal Amplification

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Table S1. Oligonucleotide sequences used in this work.

Oligonucleotide	Sequence (5' → 3')
S-SP[(a)]	AGT CCT TCC ACG ATA CCA GAT TGA CTC *GTC AGG GTA ATA AAC ACC ACG TGT GAA AGA ATT AGT GTA TCG* TGG TGT TTA TTA CCC TGA CAT AAA C
S1 RNA	AUA CAC UAA UUC UUU CAC ACG UGG UGU UUA UUA CCC UGA C
Cy5MB	Cy5-GAG TCC TTC CAC GAT ACC AGA CTC-BHQ-2
Cy5MB trigger	TGG TAT CGT GGA AGG ACT
N-SP[(a)]	CAT AGG TCT TAA CTT GTC GAT TGA CTC *CCA AAC GTA ATG CGG GGT GCA TTT CGC TGA TTT TGG GGT C*TG CAC CCC GCA TTA CGT TTG GAT GCG G
N1 RNA	GAC CCC AAA AUC AGC GAA AUG CAC CCC GCA UUA CGU UUG G
FAMMB	FAM-CCC ATA GGT CTT AAC TTG TCA TGG G-DABCYL
FAMMB trigger	GAC AAG TTA AGA CCT ATG

(a) Violet/blue, orange, red, and purple letters in SP represent trigger template, nicking endonuclease recognition site, self-priming domain, and loop of self-primed hairpin, respectively. Italic letters in SP represent the target binding region.

(b) Abbreviations: Cy5, Cyanine-5; BHQ-2, black hole quencher-2; FAM, carboxyfluorescein; DABCYL, 4-((4-(dimethylamino)phenyl)azo)benzoic Acid.

Table S2. Comparison of the SIAM method with the previous SARS-CoV-2 detection methods.

Methods	LOD[(a)] (copies/μL)	Limitations	Reference
qRT-PCR	1.25	Requirement for reverse-transcription step and thermal cycler	(Farfour et al. 2020)
AIOD-CRISPR	5	Requirement for multiple primers and CRISPR-Cas12a system	(Ding et al. 2020)
RT-LAMP-Cas12	10	Requirement for multiple primers and CRISPR-Cas12a system	(Broughton et al. 2020)
RT-LAMP	50	Requirement for multiple primers	(Rabe and Cepko 2020)
RT-LAMP	100	Requirement for multiple primers	(Baek et al. 2020)
RT-LAMP	0.2-2	Requirement for multiple primers	(Song et al. 2021)
EXPAR	72.6	False-positive signal	(Carter et al. 2021)
RT-LAMP-Cas13	25	Requirement for multiple primers and CRISPR-Cas13a system	(Agrawal et al. 2021)
COV-ID	380	Requirement for multiple primers and long preparation time	(Warneford-Thomson et al. 2022)
SIAM	0.68/1.07	Slight non-specific signal	This work

(a) LOD: limit of detection.

Table S3. Clinical sample testing using the standard qRT-PCR method.

Patient number	Sample type	qRT-PCR	
		N gene	Result
1	Nasopharyngeal swab	+	Positive
2	Nasopharyngeal swab	+	Positive
3	Nasopharyngeal swab	+	Positive
4	Nasopharyngeal swab	+	Positive
5	Nasopharyngeal swab	+	Positive
6	Nasopharyngeal swab	+	Positive
7	Nasopharyngeal swab	+	Positive
8	Nasopharyngeal swab	+	Positive
9	Nasopharyngeal swab	+	Positive
10	Nasopharyngeal swab	+	Positive
11	Sputum	+	Positive
12	Sputum	–	Negative
13	Sputum	–	Negative
14	Sputum	–	Negative
15	Sputum	–	Negative
16	Sputum	–	Negative
17	Sputum	–	Negative
18	Sputum	–	Negative
19	Sputum	–	Negative
20	Sputum	–	Negative

Table S4. Clinical testing for COVID-19 using the qRT-PCR method and the SIAM method.

Diagnostic parameter		qRT-RCR	SIAM
Positive	True	11	11
	False	-	-
Negative	True	9	9
	False	-	-
Sensitivity (%)		100	100
(95% CI[(a)]%)		(71.51 – 100)	(71.51 – 100)
Specificity (%)		100	100
(95% CI%)		(66.37 – 100)	(66.37 – 100)

[(a)] Confidence interval (MedCalc software, version 20.218)

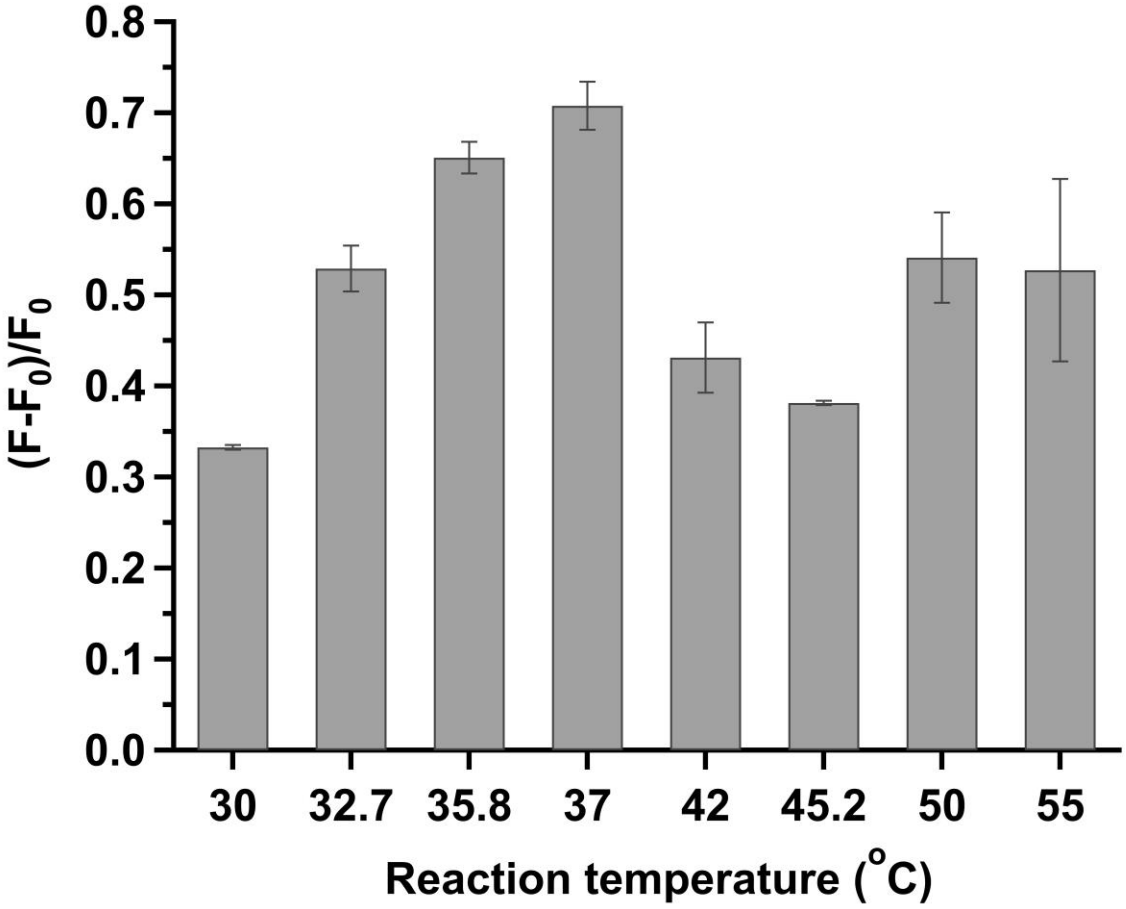

Figure S1. Optimization of reaction temperature. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction at various temperatures. Samples were incubated for 60 min. Final concentrations of SP, NE2, NE3.1, KF, NBN, MB, and N1 RNA are 20 nM, 0.4×, 0.4×, 0.5 U/μL, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

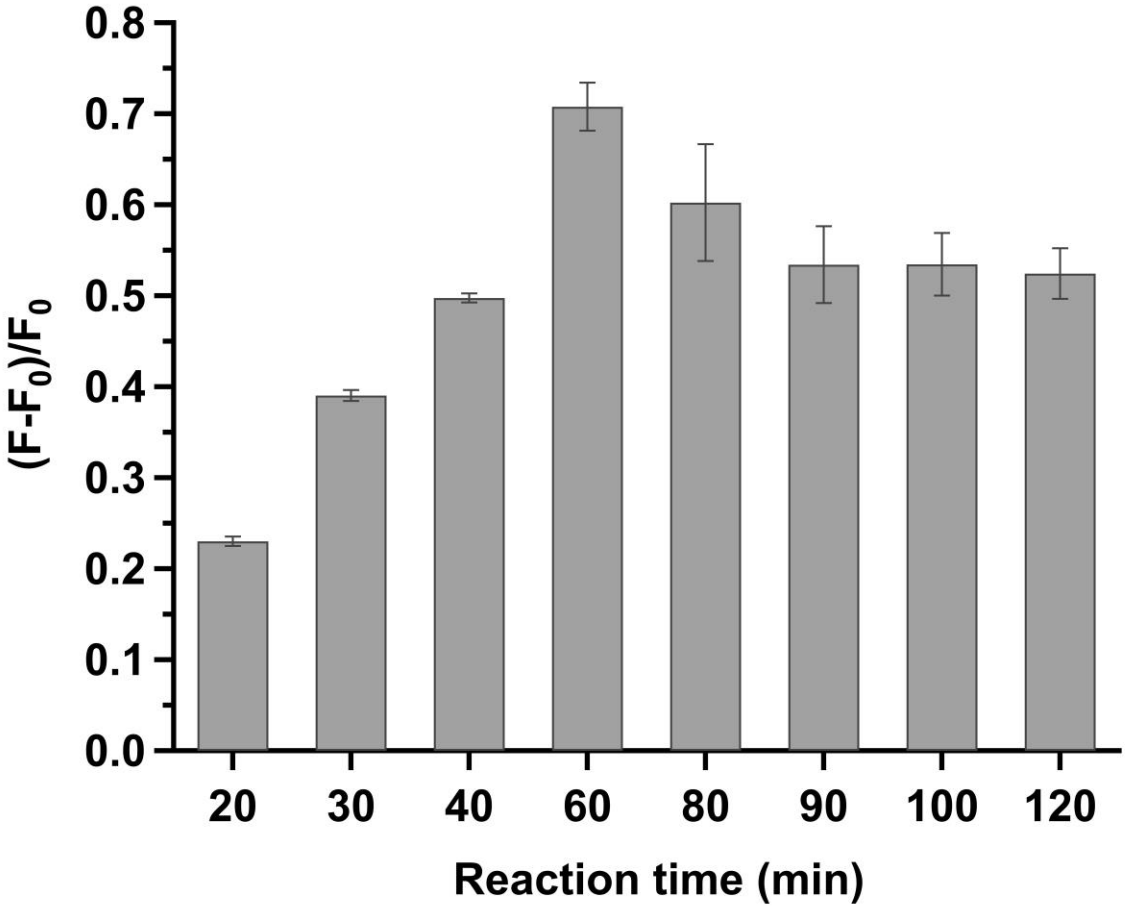

Figure S2. Optimization of reaction time. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction at various times. Samples were incubated at 37 °C. Final concentrations of SP, NE2, NE3.1, KF, NBN, MB, and N1 RNA are 20 nM, 0.4×, 0.4×, 0.5 U/μL, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

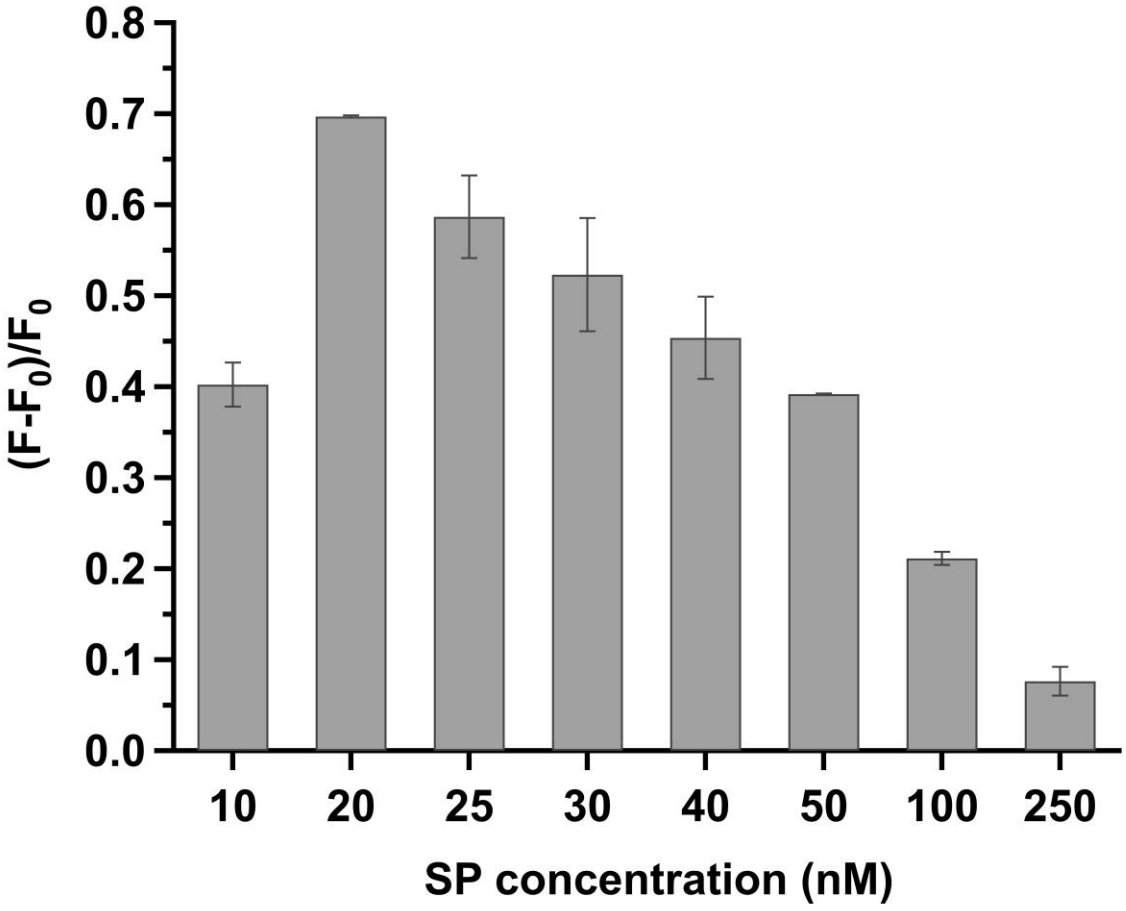

Figure S3. Optimization of SP concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of SP. Samples were incubated at 37 °C for 60 min. Final concentrations of NE2, NE3.1, KF, NBN, MB, and N1 RNA are 0.4×, 0.4×, 0.5 U/μL, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

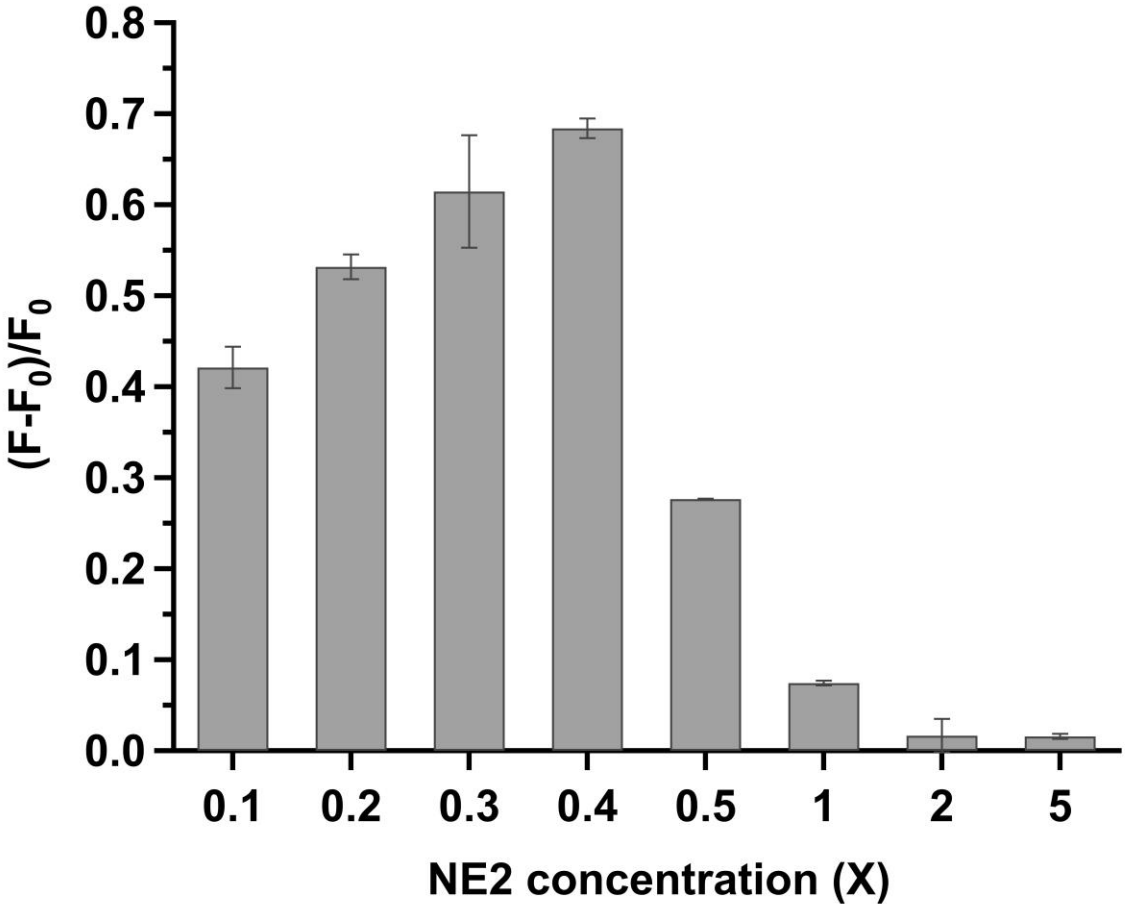

Figure S4. Optimization of NE2 concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of NE2. Samples were incubated at 37 °C for 60 min. Final concentrations of SP, NE3.1, KF, NBN, MB, and N1 RNA are 20 nM, 0.4×, 0.5 U/μL, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

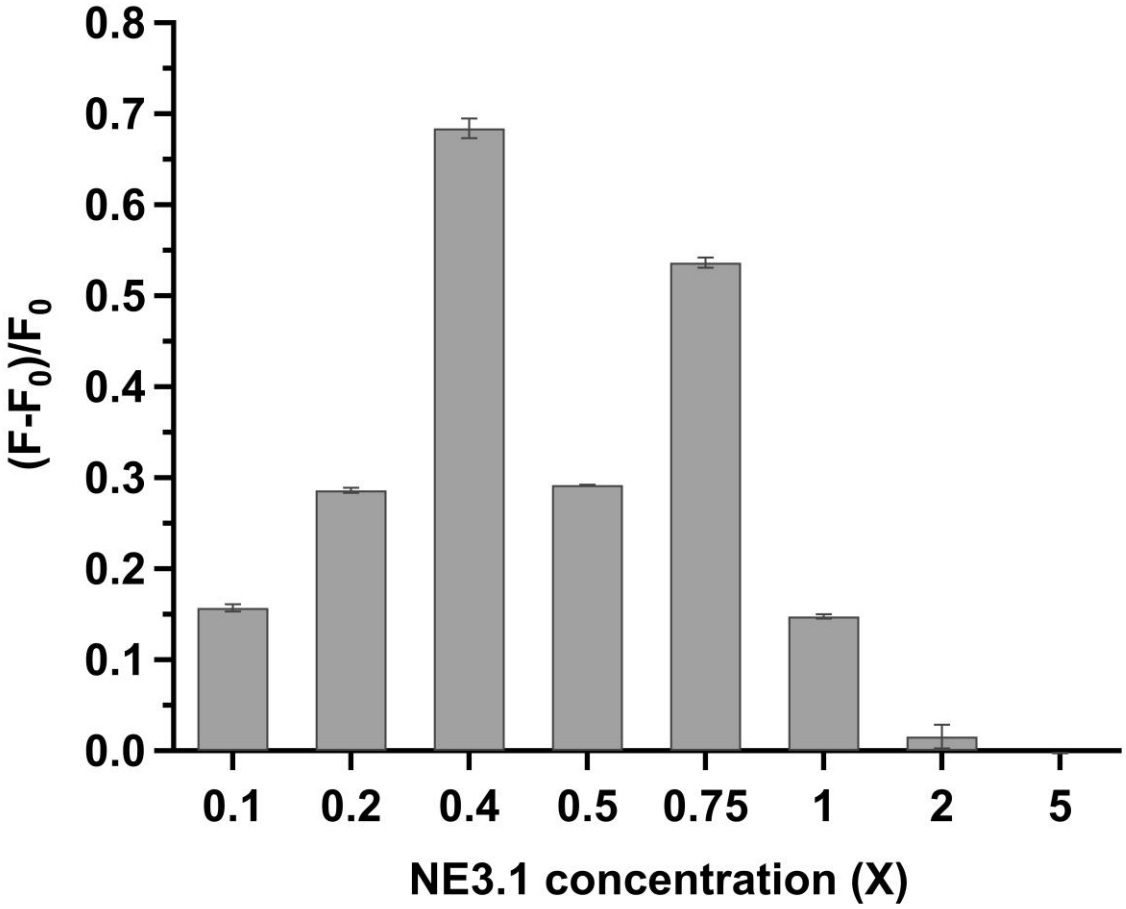

Figure S5. Optimization of NE3.1 concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of NE3.1. Samples were incubated at 37 °C for 60 min. Final concentrations of SP, NE2, KF, NBN, MB, and N1 RNA are 20 nM, 0.4×, 0.5 U/μL, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

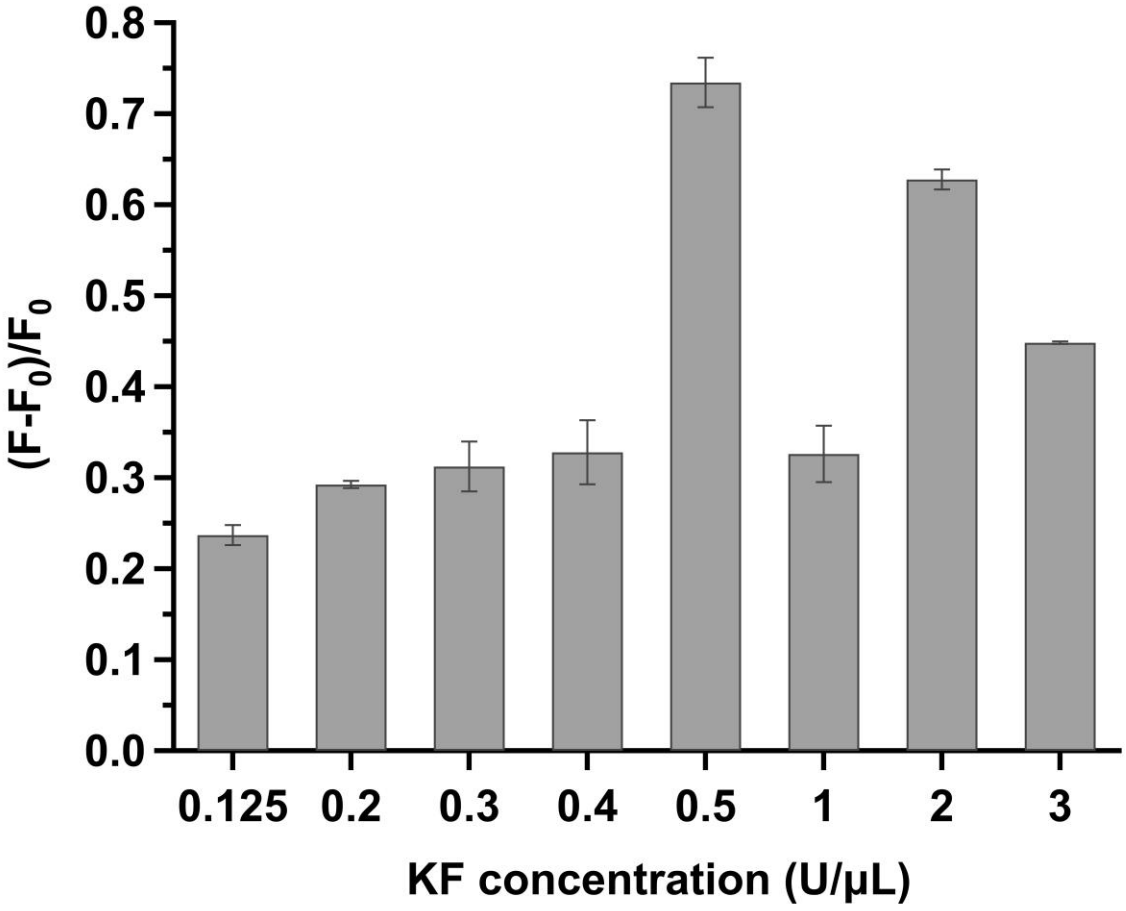

Figure S6. Optimization of KF concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of KF. Samples were incubated at 37 °C for 60 min. Final concentrations of SP, NE2, NE3.1, NBN, MB, and N1 RNA are 20 nM, 0.4×, 0.4×, 0.3 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

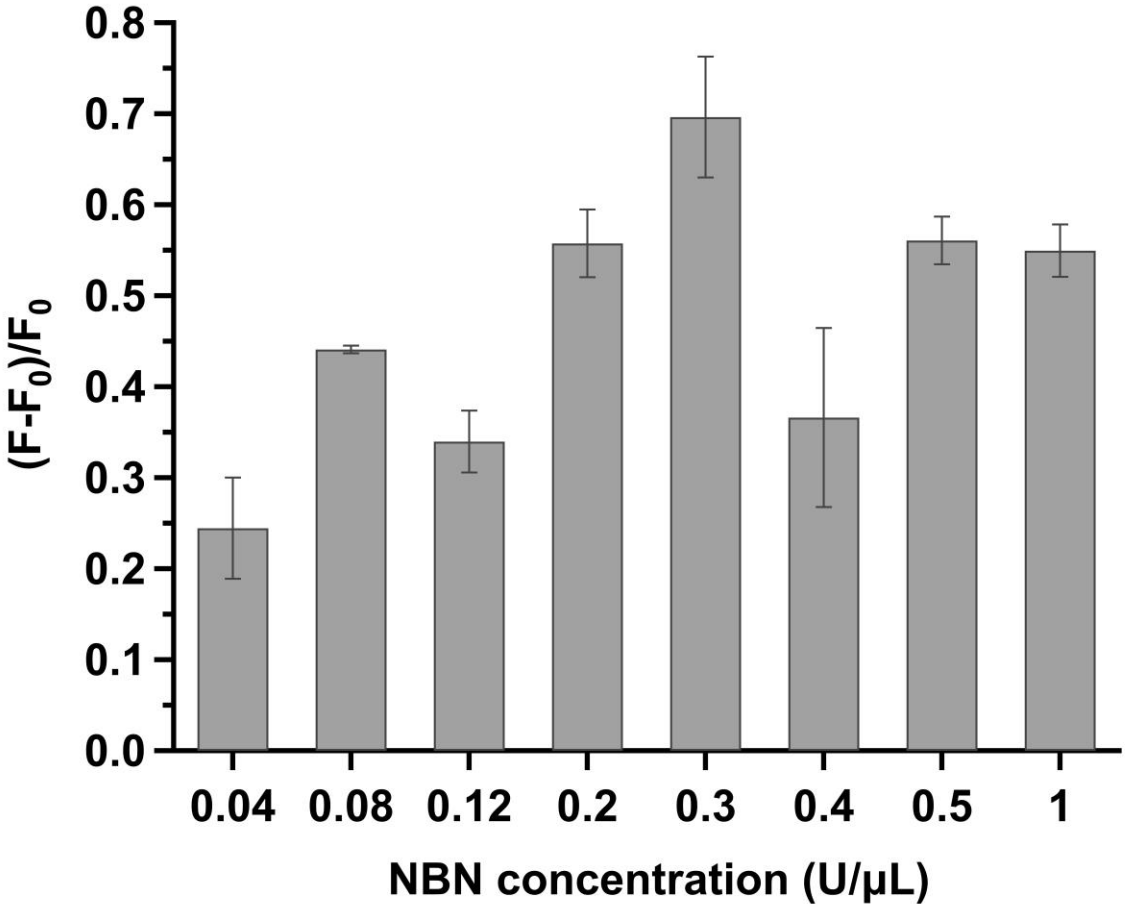

Figure S7. Optimization of NBN concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of NBN. Samples were incubated at 37 °C for 60 min. Final concentrations of SP, NE2, NE3.1, KF, MB, and N1 RNA are 20 nM, 0.4×, 0.4×, 0.5 U/μL, 300 nM, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

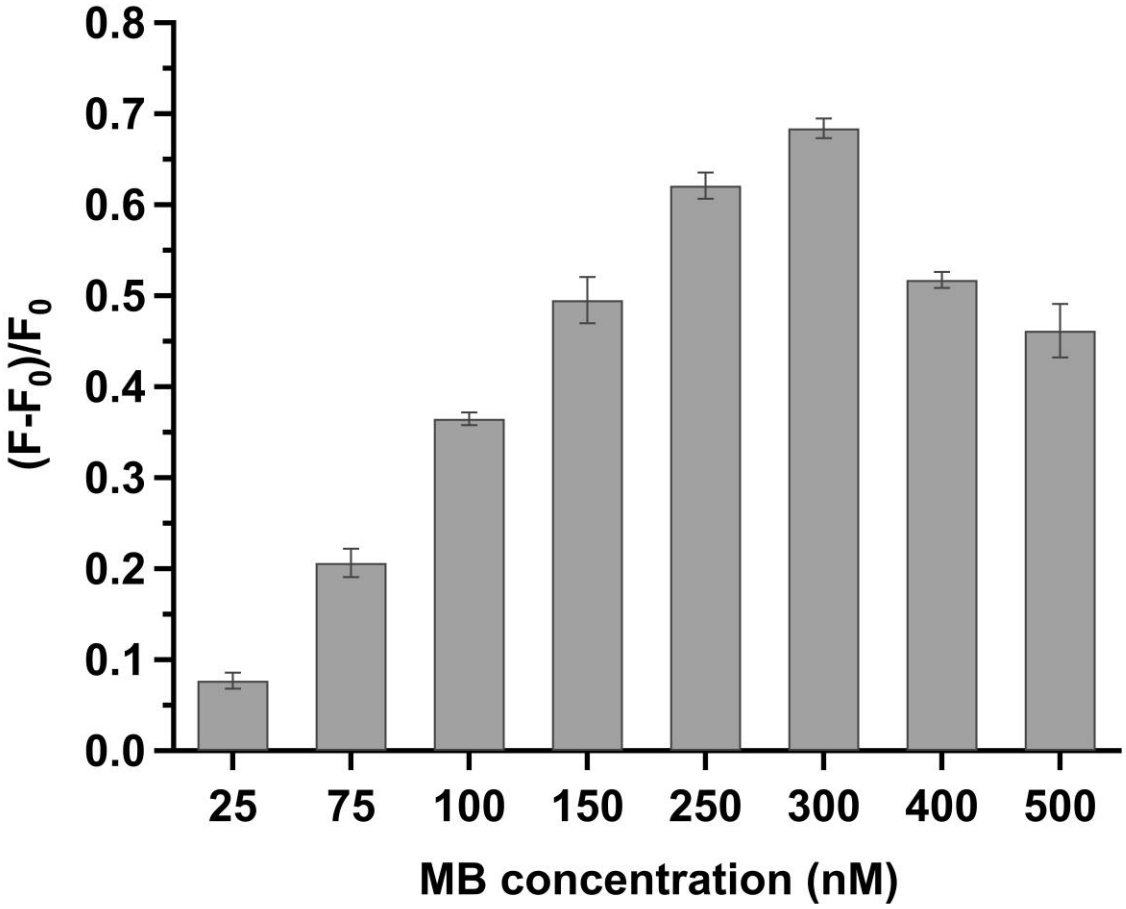

Figure S8. Optimization of MB concentration. $(F-F_0)/F_0$ of FAMMB for N1 RNA detection by the SIAM reaction using various concentrations of MB. Samples were incubated at 37 °C for 60 min. Final concentrations of SP, NE2, NE3.1, KF, NBN, and N1 RNA are 20 nM, 0.4×, 0.4×, 0.5 U/μL, 0.3 U/μL, and 1 nM, respectively. Error bars indicate the standard deviation of triplicate tests.

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