

Electronic Supplementary Material

Determination of RNase H activity via real-time monitoring of target-triggered rolling circle amplification

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Table S1 DNA sequences employed in this work.

Strand name	DNA sequence (5' → 3') ^(b)
Padlock probe ^(a)	phosphoryl- <i>GCA TCC ACT ACAC</i> CCA ACC CGC CCT ACC CAA AAC CCA ACC CGC CCT ACC CAA AAC CCA ACC <i>CGC CCT ACC CACA CTC ACC ATC</i>
Primer	TGT AGT GGA TGC GAT GGu gag uGT-amino
Control primer	TGT AGT GGA TGC GAT GGT GAG TGT-amino
Primer 1	TGT AGT GGA TGC GAT GGu gag uGT-hydroxyl
Primer 2	TGT AGT GGA TGC GAT GGu gag uGT-phosphoryl
Primer 3	TGT AGT GGA TGC GAT GGu gag uGT-sulfhydryl
Primer 4	TGT AGT GGA TGC GAT GGu gag uGT-cholesteryl
Primer 5	TGT AGT GGA TGC GAT GGu gag uGT-biotinyl

^(a) The italic letters represent the sequence complementary to that of primer.

^(b) DNA sequence is depicted in capital letters and RNA sequence is depicted in small letters.

Table S2 The fitted linear equations, slopes (V_0), mean values, and standard deviations for the data in Fig. 3(b) (n=3). The fitted linear curves were drawn in the range from 0 to 5 min for the data in Fig. 3(a) and their slopes in the linear equations were used as the initial fluorescence enhancement rates (V_0) whose mean values and standard deviations were employed in Fig. 3(b).

RNase H (U·mL ⁻¹)	Linear equation	Slope (V_0)	Mean value	Standard deviation
0	y = 8.0946x + 13.422	8.0946	8.13	0.20
	y = 8.3397x + 0.23	8.3397		
	y = 7.9463x + 4.5039	7.9463		
0.1	y = 9.6556x + 34.139	9.6556	10.33	0.71
	y = 10.259x - 6.073	10.259		
	y = 11.074x - 32.264	11.074		
0.3	y = 20.714x - 50.367	20.714	18.97	1.51
	y = 18.065x - 3.3466	18.065		
	y = 18.119x - 2.5046	18.119		
0.5	y = 25.774x - 61.896	25.774	24.33	3.20
	y = 26.552x - 41.816	26.552		
	y = 20.661x - 1.3973	20.661		
0.7	y = 29.96x - 44.716	29.96	28.62	1.73
	y = 29.227x - 15.076	29.227		
	y = 26.673x - 11.832	26.673		
1	y = 39.05x + 12.484	39.05	39.31	0.70
	y = 40.102x + 12.899	40.102		
	y = 38.785x + 19.746	38.785		
5	y = 74.435x + 15.143	74.435	70.87	6.12
	y = 74.376x - 4.2948	74.376		
	y = 63.797x - 10.084	63.797		
10	y = 112.79x + 2.6873	112.79	108.19	4.01
	y = 105.4x - 1.7683	105.4		
	y = 106.39x + 17.336	106.39		

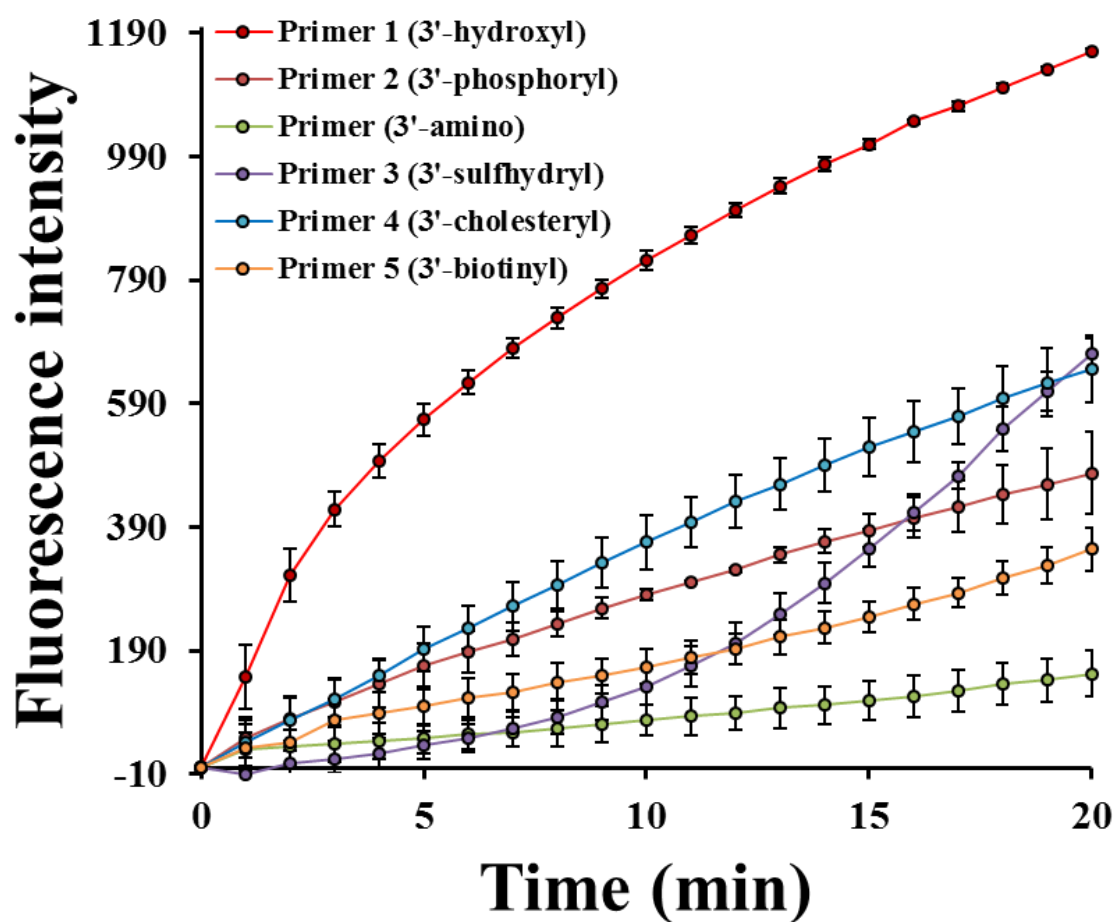
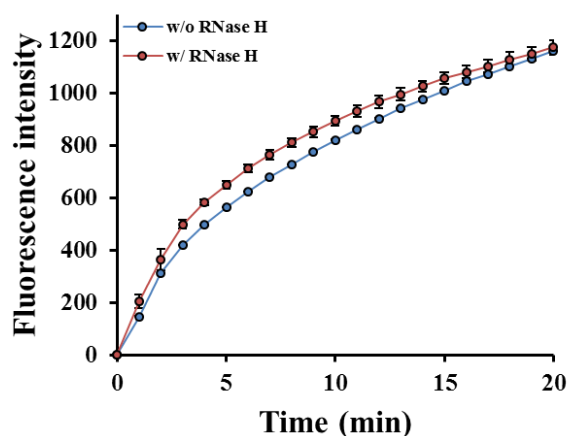
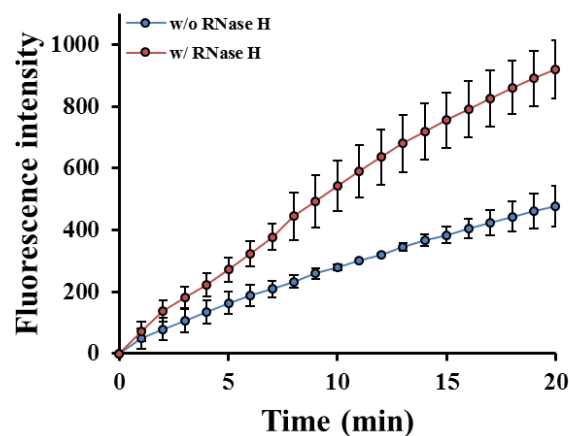


Fig. S1 The effect of different 3'-end groups of the primer on DNA polymerase activity (n=3). Time-dependent fluorescence intensities from SYBR green II during target-triggered RCA in the absence of RNase H.

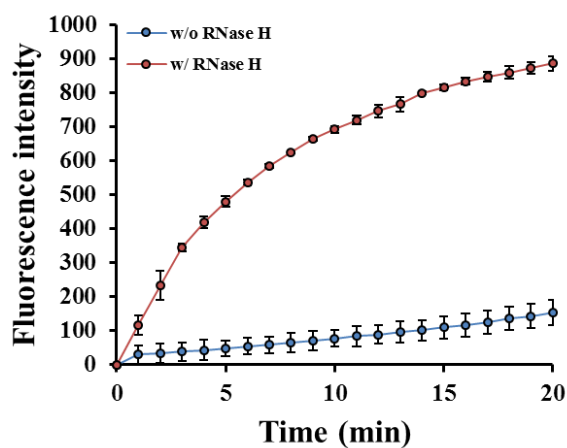
(a) Primer 1 (3'-hydroxyl)



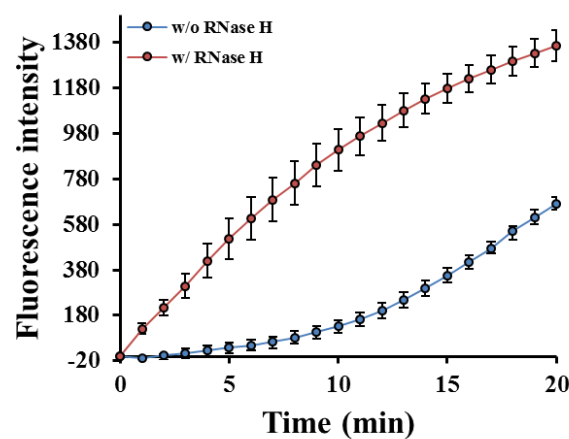
(b) Primer 2 (3'-phosphoryl)



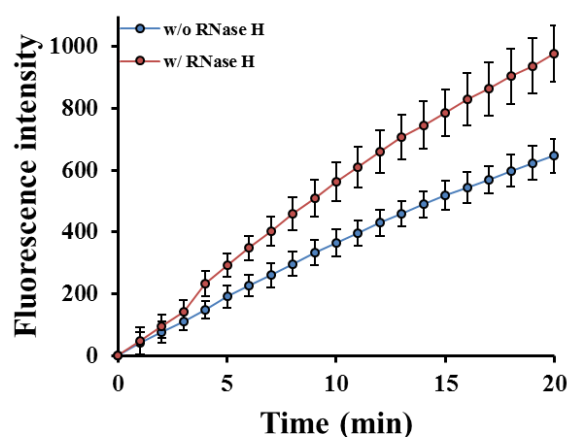
(c) Primer (3'-amino)



(d) Primer 3 (3'-sulfhydryl)



(e) Primer 4 (3'-cholesteryl)



(f) Primer 5 (3'-biotinyl)

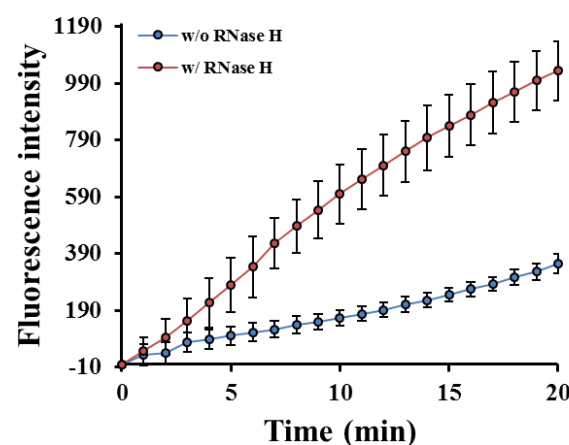


Fig. S2 The optimization of the 3'-end group of the primer for the efficient assay of RNase H activity ($n=3$). Time-dependent fluorescence intensities from SYBR green II during target-triggered RCA in the absence and presence of RNase H ($10 \text{ U} \cdot \text{mL}^{-1}$). From (a) to (f), primers with different 3'-end group are used, which is indicated in parentheses.