RNase H2-dependent PCR (rhPCR)

REVISION HISTORY

Version	Release date	Description of changes
2	November 2022	Updated to internal MAPSS standards.
1	September 2017	Initital release.

Table of contents

OVERVIEW

The procedure for rhPCR is similar to standard qPCR, but requires blocked-cleavable rhPCR primers (rhPrimers) and the addition of RNase H2 enzyme to the master mix. The *Pyrococcus abyssi* (*P.a.*) RNase H2 is thermostable and will continue to function throughout PCR cycling.

P.a. RNase H2 is available at 2 concentrations, 20 U/µL and 2 U/µL. The enzyme must be diluted before use. **Please use the Enzyme Dilution Buffer that is supplied with the enzyme.**

REACTION RECOMMENDATIONS

GEN1—use 2–10 mU RNase H2 for 10 µL reactions that contain rhPrimers GEN1. For larger reactions, the amount of enzyme used should be scaled up proportionally (e.g., use 5–25 mU RNase H2 for 25 µL reactions). Start with 5 mU/10 µL (0.5 mU/µL) and titrate the enzyme up or down so that reaction efficiency is similar to control reactions set up using unmodified primers. Using insufficient enzyme will lower reaction efficiency and will require additional PCR cycles. Excess enzyme will decrease precision, removing the benefit of performing rhPCR.

GEN2—use 5–300 mU RNase H2 per 10 µL reaction mix for reactions that contain rhPrimers GEN2. If one of the primers contains an rU residue at the cleavage site, it might be necessary to use more enzyme than for primers that contain rA, rG, or rC residues. As with GEN1 primers, titrate the amount of RNase H2 needed.

Tip: Use more RNase H2 for multiplex reactions than for single plex reactions. The precise amount needed for a multiplex reaction varies with the number of amplicons being detected, primer concentration, and buffer composition. We recommend that you optimize the reaction by testing a variety of enzyme concentrations.

Note: Ensure a minimum final concentration of 0.01% Triton X-100 (or equivalent non-ionic detergent) in the reaction. The enzyme dilution buffer provided with the RNase H2 contains 0.1% Triton X-100. Therefore, if a 10X stock enzyme solution is used, simply add the enzyme in a 1:10 ratio into the reaction mix to achieve the correct Triton X-100 concentration. The reaction will not be affected if detergent levels are 2–3X above the recommended minimum, but reaction efficiency decreases if detergent drops below this level.

Table 1. Example of a homemade rhPCR mix for rhPrimers GEN1.

Component	Final concentration
Tris pH 8.4	20 mM
KCl	50 mM
$MgCl_2$	3.0 mM
dNTPs	0.8 mM (0.2 mM each)
Triton X-100	0.01%
Forward primer	200 nM
Reverse primer	200 nM
Target DNA	(variable)
Taq polymerase	0.5 U
P.a. RNase H2	0.5 mU/µL (1 µL of a 5 mU/µL stock solution)
Final volume	**10 µL**

PCR design considerations

IDT recommends use of a 2-step PCR cycle. During PCR, the blocked-cleavable primers anneal to the target and are activated (cleaved by RNase H2) during the anneal phase of the reaction. In a 2-step PCR cycle, the anneal phase also serves as the polymerase extend phase, so this phase is longer and allows for the highest amount of primer activation. In a 3-step PCR cycle, following a short primer anneal step (usually done at 60°C), a higher temperature (such as 72°C) is used for the polymerase extend step. At this higher temperature, primer annealing and activation do not occur, making a 3-step PCR less efficient. If a 3-step PCR is desired, increasing the length of the primer annealing step or increasing the RNase H2 concentration can compensate. Alternatively, 2-step PCR can be performed at higher temperature, which will require making the primers longer to increase their T_m to the desired reaction temperature (such as 72°C).

Note: Higher amounts of RNase H2 may be needed for reactions performed at temperatures below 55°C. *P.a.* RNase H2 has highest activity in the range of 60–70°C. Using higher amounts of RNase H2 allows annealing/extension times to be decreased, while decreasing the amount of RNase H2 requires extending the duration of annealing/extension times.

COMPATIBILITY WITH COMMERCIALLY AVAILABLE PCR MIXES

IDT scientists have tested the compatibility of *P.a.* RNase H2 and rhPCR with many commercial PCR master mixes using manufacturers' recommended cycling conditions (Tables 2, 4, and 6) and IDT "standard" cycling conditions* (Tables 3, 5, and 7). Commercial polymerases have also been tested (Table 10). Most master mixes and polymerases perform well; however, we recommend empirical testing of each mix or polymerase because buffer composition affects reaction results.

In general, high-fidelity polymerases with 3'-exonuclease activity perform poorly with rhPrimers GEN1 (rDDDDMx) because the exonuclease function removes the 3' blocking group, allowing amplification to occur in the absence of RNase H2 and removing the benefits of using blocked-cleavable primers. However, the 3' block for rhPrimers GEN2 (rDxxDM) is more stable, so these are compatible with high fidelity 3'-exo polymerases.

* IDT standard cycling conditions: 3 min, 95°C; 45 x (10 sec, 95°C; 30 sec, 60°C)

APPENDIX A: COMPATIBILITY OF rhPRIMERS GEN1 WITH COMMERCIAL MASTER MIXES

Table 2. Compatibility of rhPrimers GEN1 (rDDDDMx) with commercial master mixes using manufacturers' cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL reaction (mU)	Notes
Applied Biosystems TaqMan® Fast Advanced	5	
Applied Biosystems TaqMan Gene Expression	1.3	
Bio-Rad iTaq™ DNA Polymerase	1.3	
Bio-Rad iQ™ Multiplex Powermix	2.6	Low quality amplification in rhPCR
Bio-Rad SsoFast™ Probes Supermix	5	Low quality amplification in rhPCR
Invitrogen EXPRESS qPCR Supermix	1.3	
Kapa Probe Fast qPCR	5	
PCR Biosystems qPCRBIO Probe Mix Lo-ROX	5	
Quantabio PerfeCTa™ Multiplex qPCR SuperMix	2.6	
Qiagen Multiplex PCR Plus Kit	Not recommended	Low quality amplification in rhPCR
Qiagen QuantiTect® Multiplex PCR Kit	2.6	
Roche FastStart™ TaqMan Probe Master	Not recommended	Low quality amplification in rhPCR

Table 3. Compatibility of rhPrimers GEN1 (rDDDDMx) with commercial master mixes using IDT standard cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL Reaction (mU)	Notes
Applied Biosystems TaqMan Fast Advanced	5	
Applied Biosystems TaqMan Gene Expression	2.6	
Bio-Rad iTaq DNA Polymerase	2.6	
Bio-Rad iQ Multiplex Powermix	2.6	
Bio-Rad SsoFast Probes Supermix	2.6	
Invitrogen EXPRESS qPCR Supermix	2.6	
Kapa Probe Fast qPCR	2.6	
PCR Biosystems qPCRBIO Probe Mix Lo-Rox	2.6	
Quantabio PerfeCTa Multiplex qPCR SuperMix	1.3	
Qiagen Multiplex PCR Plus Kit	Not recommended	Low quality amplification in rhPCR
Qiagen QuantiTect Multiplex PCR Kit	2.6	
Roche FastStart TaqMan Probe Master	Not recommended	Low quality amplification in rhPCR

APPENDIX B: COMPATIBILITY OF rhPRIMERS GEN2 WITH COMMERCIAL MASTER MIXES

Table 4. Compatibility of rhPrimers GEN2 (rDxxDM) with commercial master mixes using manufacturers' cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL reaction (mU)	Notes
Applied Biosystems TaqMan Gene Expression	100	
Bio-Rad iTaq DNA Polymerase	200	
Bio-Rad iQ Multiplex Powermix	150	
Bio-Rad SsoFast Probes Supermix	400	
Invitrogen EXPRESS qPCR Supermix	100	
Kapa Probe Fast qPCR	100	
PCR Biosystems qPCRBIO Probe Mix Lo-Rox	100	
Quantabio PerfeCTa Multiplex qPCR SuperMix	100	
Qiagen Multiplex PCR Plus Kit	Not recommended	Low quality amplification in rhPCR
Qiagen QuantiTect Multiplex PCR Kit	200	

* This survey was performed using a forward primer with rUDxxD design and an unmodified reverse primer. If neither primer contains a rU residue, the amount of RNase H2 required to achieve peak cycling efficiency will be lower.

Table 5. Compatibility of rhPrimers GEN2 (rDxxDM) with commercial master mixes using IDT standard cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL reaction (mU)	Notes
Applied Biosystems TaqMan Gene Expression	150	
Bio-Rad iTaq DNA Polymerase	200	
Bio-Rad iQ Multiplex Powermix	150	
Bio-Rad SsoFast Probes Supermix	150	
Invitrogen EXPRESS qPCR Supermix	100	
Kapa Probe Fast qPCR	150	
PCR Biosystems qPCRBIO Probe Mix Lo-Rox	100	
Quantabio PerfeCTa Multiplex qPCR SuperMix	100	
Qiagen Multiplex PCR Plus Kit	Not recommended	Low quality amplification in rhPCR
Qiagen QuantiTect Multiplex PCR Kit	200	

* This survey was performed using a forward primer with rUDxxD design and an unmodified reverse primer. If neither primer contains a rU residue, the amount of RNase H2 required to achieve peak cycling efficiency will be lower.

APPENDIX C: COMPATIBILITY OF rhPRIMERS GEN1 WITH INTERCALATING DYE-BASED MASTER MIXES

Table 6. Compatibility of rhPrimers GEN1 (rDDDDMx) with intercalating dye-based master mixes using manufacturers' cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL reaction (mU)
Bio-Rad SsoAdvanced™ SYBR® Green	50
Bio-Rad SsoFast EvaGreen®	50
Bio-Rad SYBR Fast qPCR	5

Table 7. Compatibility of rhPrimers GEN1 (rDDDDMx) with intercalating dye-based master mixes using IDT standard cycling conditions.

Master mix	Amount of RNase H2 required per 10 µL reaction (mU)
Bio-Rad SsoAdvanced SYBR Green	50
Bio-Rad SsoFast EvaGreen	50

APPENDIX D: COMPATIBILITY OF rhPRIMERS GEN1 WITH SYBR® GREEN DYE-BASED MASTER MIXES

Table 8. Compatibility of rhPrimers GEN1 (rDDDDMx) with SYBR Green dye-based and similar master mixes.

Master mix	Relative quality of rhPCR amplification	Amount of RNase H2 required per 10 µL reaction (mU)
Agilent Brilliant SYBR Green	++	2.6
Agilent Brilliant II SYBR Green	++	2.6
Applied Biosystems SYBR Green PCR	++	2.6
Bio-Rad iQ SYBR Green Supermix	++	2.6
Biotium Fast EvaGreen qPCR	++	2.6
Invitrogen Platinum™ SYBR Green qPCR SuperMix-UDG	++	2.6
Promega GoTaq® Green	++	2.6
Qiagen HotStarTaq® Plus + Invitrogen SYBR GreenER™	++	2.6
Qiagen QuantiTect SYBR Green PCR Kit	++	2.6
Roche LightCycler® 480 SYBR Green I	++	2.6

APPENDIX E: COMPATIBILITY OF rhPRIMERS GEN2 WITH SYBR GREEN DYE-BASED MASTER MIXES

Table 9. Compatibility of rhPrimers GEN2 (rDxxDM) with SYBR Green dye-based and similar master mixes.

Master mix	Relative quality of rhPCR amplification	Amount of RNase H2 required per 10 µL reaction (mU)
Agilent Brilliant SYBR Green	++	100
Agilent Brilliant II SYBR Green	++	50
Applied Biosystems SYBR Green PCR	++	100
Bio-Rad iQ SYBR Green Supermix	++	200
Biotium Fast EvaGreen qPCR	++	200
Invitrogen Platinum SYBR Green qPCR SuperMix-UDG	++	50
Promega GoTaq Green	++	200
Qiagen HotStarTaq Plus + Invitrogen SYBR GreenER	++	50
Qiagen QuantiTect SYBR Green PCR Kit	++	>200
Roche LightCycler 480 SYBR Green I	++	100

APPENDIX F: COMPATIBILITY OF rhPRIMERS GEN1 WITH VARIOUS POLYMERASES

Important: High-fidelity polymerases are not recommended for use with rhPrimers.

Table 10. Compatibility of rhPrimers GEN1 (rDDDDMx) with various polymerases.

DNA polymerase	Relative quality of rhPCR amplification	Amount of RNase H2 required per 10 µL reaction (mU)	Notes*
Enzymatics Taq	++	1.3	
iTaq	++	1.3	
Tfl DNA polymerase	++	2.6	
Titanium™ Taq (Takara)	++	1.3	
HotStarTaq	++	2.6	
Amplitaq Gold™ (Applied Biosystems)	++	2.6	
Amplitaq™ (Applied Biosystems)	++	2.6	
DyNAzyme II Hot Start	++	1.3	
FastStart™ Taq (Roche)	++	1.3	
Deep Vent® (exo-) (New England BioLabs)	++	2.6	
Vent® (exo-) (New England BioLabs)	++	2.6	
Immolase™ (Bioline)	++	10	
KOD Hot Start	++	10	
Tli DNA polymerase	+	10	Inconsistent results
PfuUltra™ HF (Agilent)	–		No amplification
Phusion ™ (Thermo Fisher Scientific)	–		Blocked primers are cleaved without RNase H2
Phire™ Hot Start (Thermo Fisher Scientific)	–		>10 mU RNase H2 required
Deep Vent® (New England BioLabs)	–		Blocked primers are cleaved without RNase H2
Vent® (New England BioLabs)	–		Blocked primers are cleaved without RNase H2
9°N$_m$™ (New England BioLabs)	–		Unusual amplification curves; not recommended

++ = good compatibility; + = compatible, but may require more RNase H2; – = not compatible

* The buffer recommended for each polymerase was used. Some polymerases may work with rhPCR if the buffer composition is modified. However, because most polymerase vendors do not provide buffer composition, it is difficult to adjust individual components.

RNase H2-dependent PCR (rhPCR)

For more information, go to: **www.idtdna.com/ContactUs**

> For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

Doc ID: RUO22-1479_001 02/23