

## Watchmaker Stellar qPCR and RT-qPCR Kits

### Product Description

The Watchmaker Stellar qPCR and RT-qPCR portfolio consists of two kits:

- The **Watchmaker Stellar qPCR Kit** contains StellarTaq™ Hot Start DNA Polymerase (StellarTaq HS) for the rapid amplification of DNA targets in multiplex probe-based qPCR as well as two different 10X reaction buffers and 25 mM MgCl<sub>2</sub>.
- The **Watchmaker Stellar RT-qPCR Kit** contains StellarScript® HT+ Reverse Transcriptase (StellarScript HT+) and StellarTaq HS for the rapid detection of RNA targets using one-step multiplex probe-based RT-qPCR as well as two different 10X reaction buffers and 25 mM MgCl<sub>2</sub>.

The Stellar qPCR and RT-qPCR Kits were developed for multiplex probe-based qPCR or RT-qPCR directly in samples or in the presence of inhibitors that may be carried over during DNA purification. Other applications, such as intercalating dye-based qPCR and endpoint PCR can also be carried out using this system.

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### Kit Contents

Kit	Kit code	Description	Component volume
Stellar qPCR Kit	<b>7K0143-500</b>	StellarTaq Hot Start DNA Polymerase (5 U/μL)	200 μL
		10X Stellar Reaction Buffer A	1 mL
		10X Stellar Reaction Buffer B	1 mL
		25 mM MgCl <sub>2</sub>	4 mL
Stellar RT-qPCR Kit	<b>7K0142-500</b>	StellarTaq Hot Start DNA Polymerase (5 U/μL)	200 μL
		StellarScript HT+ Reverse Transcriptase (200 U/μL)	1 mL
		10X Stellar Reaction Buffer A	1 mL
		10X Stellar Reaction Buffer B	1 mL
		25 mM MgCl <sub>2</sub>	4 mL

For custom formats, contact the **Sales Team** at [sales@watchmakergenomics.com](mailto:sales@watchmakergenomics.com).

## Product Description (continued)

StellarTaq HS has been engineered for speed, extreme inhibitor tolerance, and specificity. The polymerase catalyzes 5'→3' DNA synthesis, has 5'→3' exonuclease activity, and is deficient in 3'→5' exonuclease activity making it suitable for probe digestion. StellarTaq HS amplifies uracil-containing templates, incorporates modified bases, and performs A-tailing of DNA products. The hot start mechanism inhibits 5'→3' polymerase activity and 5'→3' exonuclease activity, ensuring high target specificity even when amplification reactions are set up at room temperature.

StellarScript HT+ is a M-MLV variant which has been engineered for increased thermostability and inhibitor tolerance. It also has reduced RNase H activity. This enzyme has optimal activity between 42°C and 65°C, providing increased specificity, higher cDNA yields, and longer cDNA products than wild-type M-MLV RT.

The Stellar qPCR and RT-qPCR Kits are supplied with two different 10X reaction buffers, (10X Stellar Reaction Buffers A and B) to facilitate efficient and rapid amplification in a wide range of different samples. Both kits are also supplied with the ancillary component, 25 mM MgCl<sub>2</sub>.

Alternative formats of StellarTaq HS (including glycerol-free versions – see [StellarTaq DNA Polymerase Technical Guide](#)) and StellarScript HT+ are available to pair with the Stellar qPCR and RT-qPCR Kit components. Contact [sales@watchmakergenomics.com](mailto:sales@watchmakergenomics.com) for information.

## Relevant Stellar qPCR and RT-qPCR Kit Applications\*

- Multiplex probe-based qPCR
- Multiplex probe-based RT-qPCR<sup>1</sup>
- Pathogen detection, including infectious diseases<sup>2,3</sup>
- Intercalating dye-based qPCR<sup>4</sup>
- PCR applications where inhibitors are present<sup>5</sup>
- Fast PCR<sup>6</sup>
- PCR applications where specificity is important<sup>7</sup>
- PCR amplification of DNA fragments (≤5 kb)

\*Watchmaker Genomics has not tested or validated StellarTaq HS or StellarScript HT+ in all applications listed.

## Unit Definition and Buffer Composition

- 1 unit of StellarTaq HS incorporates 16 nmol of dNTPs into a DNA template in 30 minutes at 72°C.
- 1 unit of StellarScript HT+ incorporates 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using poly r(A)/oligo (dT) as a substrate.
- Storage buffers:
  - StellarTaq HS: 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 0.1 mM EDTA, 50% Glycerol, 0.05% Tween 20
  - StellarScript HT+: 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 50% Glycerol, 0.1% Tween 20

## Storage and Handling

- The Stellar qPCR and RT-qPCR Kits are shipped on ice packs or dry ice. Upon receipt, store all kit components at -20°C ± 5°C.
  - Glycerol-containing components (StellarTaq HS and StellarScript HT+) will not freeze at -25°C to -15°C, but will freeze if shipped on dry ice or stored at -70°C to -90°C.
- If ordered separately, glycerol-free versions of StellarTaq HS will be shipped on dry ice and should be stored at -70°C to -90°C.

Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to mix solutions thoroughly before use and during reaction setup. Do not vortex the polymerase. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

## Recommended Reaction Optimization for Stellar qPCR and RT-qPCR

### *10X Stellar Reaction Buffers*

The 10X Stellar Reaction Buffers A and B have different properties and may perform better with a chosen set of primers, cycling conditions, or inhibitors that may be present in the sample. We recommend doing a trial amplification using each of the buffers individually in the assay, and subsequently selecting the better performing buffer for future assays.

**NOTE: Do not** use both buffers in the same amplification reaction. Use **either** 10X Stellar Reaction Buffer A **or** 10X Stellar Reaction Buffer B in a single reaction.

### *dNTPs*

dNTPs are not supplied with the Watchmaker qPCR or RT-qPCR Kits. For optimal performance, use high-quality dNTPs which have been HPLC-purified ( $\geq 99\%$  purity) from a quality supplier such as Larova GmbH.

### *Reference dyes and contamination controls*

The Stellar qPCR and RT-qPCR Kits are compatible with:

- *Reference dyes:* Reference dyes are not included in the Watchmaker qPCR or RT-qPCR Kits. Refer to your qPCR instrument for instructions on which reference dye type and what concentration to use.
- *Uracil-DNA Glycosylase (UNG):* UNG is not included in the Watchmaker qPCR or RT-qPCR Kits. For carryover contamination control with UNG, the ratio of dTTP and dUTP must be optimized. A recommended starting concentration of 20% dTTP and 80% dUTP is recommended.

## Recommended Reaction Setup

The following reaction setup tables provide initial final concentration/volume recommendations for multiplex probe-based qPCR (1) and RT-qPCR (2) carried out in a total amplification reaction volume of 20  $\mu\text{L}$ . For recommended concentrations for intercalating dye-based qPCR and endpoint PCR, refer to **Appendix A1**. For intercalating dye-based RT-qPCR or endpoint RT-PCR, refer to **Appendix A2**. Guidance for further optimization of reactions is outlined in **Appendix B – Troubleshooting Guide**.

### 1. Multiplex probe-based qPCR

Component	Final concentration	Volume (20 $\mu\text{L}$ reaction)
10X Stellar Reaction Buffer A or B	1X	2 $\mu\text{L}$
25 mM $\text{MgCl}_2^a$	4 mM (2 – 8 mM)	3.2 $\mu\text{L}$
10 $\mu\text{M}$ Primers (Forward and Reverse) <sup>b</sup>	0.4 $\mu\text{M}$ (0.2 – 0.6 $\mu\text{M}$ )	0.8 $\mu\text{L}$
10 $\mu\text{M}$ Probe(s) <sup>b</sup>	0.2 $\mu\text{M}$ (0.1 – 0.6 $\mu\text{M}$ )	0.4 $\mu\text{L}$
StellarTaq DNA Polymerase (5 U/ $\mu\text{L}$ ) <sup>c</sup>	0.05 U/ $\mu\text{L}$ (0.012 – 0.096 U/ $\mu\text{L}$ )	0.2 $\mu\text{L}$
dNTPs (10 mM each) <sup>b</sup>	0.2 mM	0.4 $\mu\text{L}$
Template DNA <sup>d</sup>	Variable	Variable
PCR-grade water <sup>b</sup>	–	Up to 20 $\mu\text{L}$

### 2. Multiplex probe-based RT-qPCR

Component	Final concentration	Volume (20 $\mu\text{L}$ reaction)
10X Stellar Reaction Buffer A or B	1X	2 $\mu\text{L}$
25 mM $\text{MgCl}_2^a$	4 mM (2 – 8 mM)	3.2 $\mu\text{L}$
10 $\mu\text{M}$ Primers (Forward and Reverse) <sup>b</sup>	0.4 $\mu\text{M}$ (0.2 – 0.6 $\mu\text{M}$ )	0.8 $\mu\text{L}$
10 $\mu\text{M}$ Probe(s) <sup>b</sup>	0.2 $\mu\text{M}$ (0.1 – 0.6 $\mu\text{M}$ )	0.4 $\mu\text{L}$
StellarTaq DNA Polymerase(5 U/ $\mu\text{L}$ ) <sup>c</sup>	0.05 U/ $\mu\text{L}$ (0.012 – 0.096 U/ $\mu\text{L}$ )	0.2 $\mu\text{L}$
StellarScript HT+ Reverse Transcriptase (200 U/ $\mu\text{L}$ ) <sup>e</sup>	1.2 U/ $\mu\text{L}$ (0.4 – 3.6 U/ $\mu\text{L}$ )	0.12 $\mu\text{L}$
dNTPs (10 mM each) <sup>b</sup>	0.2 mM	0.4 $\mu\text{L}$
Template RNA <sup>d</sup>	Variable	Variable
RNase Inhibitor (40 U/ $\mu\text{L}$ ) <sup>b</sup> (Optional) <sup>f</sup>	1 U/ $\mu\text{L}$	0.5 $\mu\text{L}$
PCR-grade water <sup>b</sup>	–	Up to 20 $\mu\text{L}$

<sup>a</sup> The  $\text{MgCl}_2$  concentration may need to be optimized.

<sup>b</sup> Not supplied in the Watchmaker Stellar qPCR or RT-qPCR Kits.

<sup>c</sup> Use 0.05 U/ $\mu\text{L}$  as a final StellarTaq HS concentration for multiplex (up to 5 targets) probe-based (RT)-qPCR. Use lower enzyme concentrations, down to 0.012 U/ $\mu\text{L}$ , for single target probe-based (RT)-qPCR if non-specific amplification is observed. Increase enzyme concentration, up to 0.096 U/ $\mu\text{L}$ , if PCR inhibition is observed.

<sup>d</sup> Template DNA/RNA input is assay-dependent and must be optimized.

<sup>e</sup> 1.2 U/ $\mu\text{L}$  of StellarScript HT+ is a good starting concentration. Use lower concentrations, down to 0.4 U/ $\mu\text{L}$ , for reactions that do not contain inhibitors. Use higher concentrations, up to 3.6 U/ $\mu\text{L}$ , for reactions that contain inhibitors.

<sup>f</sup> Watchmaker Genomics RNase Inhibitor (40 U/ $\mu\text{L}$ ) is not included in the qPCR and RT-qPCR Kits but is supplied separately by Watchmaker Genomics. RNase Inhibitor can be included in the reaction to prevent RNA degradation in samples containing RNases.

### Recommended Cycling Protocol

Recommended cycle protocols are shown below for qPCR and RT-qPCR. Cycle protocols may require optimization depending on sample type and input amount, primer sequences, or target length. Refer to **Appendix B – Troubleshooting Guide** for optimization.

#### 1. qPCR

Step	Temperature (°C)	Time	Cycles
Initial denaturation <sup>†</sup>	95	1:00	1
Denaturation	95	0:05	25 – 50 <sup>‡‡</sup>
Annealing and extension <sup>††</sup>	~60 <sup>‡</sup>	0:10	
–	12	Hold	–

#### 2. RT-qPCR

Step	Temperature (°C)	Time	Cycles
Reverse transcription	55	3:00	1
Initial denaturation <sup>†</sup>	95	1:00	1
Denaturation	95	0:05	25 – 50 <sup>‡‡</sup>
Annealing and extension <sup>††</sup>	~60 <sup>‡</sup>	0:10	
–	12	Hold	–

<sup>†</sup>Initial denaturation time depends on sample type. A 60-second denaturation time should be used as a first approach. Complex samples may require extended denaturation time (3 – 5 minutes). The initial denaturing could be reduced to as short as 10 seconds as the Hot Start antibody only requires 10 seconds at 95°C to allow full activation of the enzyme.

<sup>††</sup>A 10-second combined annealing and extension time is appropriate for 4 or 5 multiplexed targets (≤150 bp). Targets ≥1000 bp in length may require increased extension times. Applications requiring fast PCR can be optimized by decreasing the combined annealing and extension time and/or by increasing the enzyme concentration. Traditional three-step PCR can also be performed.

<sup>‡</sup>The annealing temperature is dependent on the primer(s) sequence(s). Optimal annealing temperature is determined empirically in an annealing temperature gradient experiment across a range of 55°C to 72°C.

<sup>‡‡</sup>Cycle number varies based on DNA input amount.

## Appendix A

### 1. Recommended reaction set up for intercalating dye-based qPCR and endpoint PCR

Component	Final concentration	
	Intercalating dye-based qPCR	Endpoint PCR
10X Stellar Reaction Buffer A or B	1X	
Intercalating dye (e.g., 500X SYBR) <sup>a</sup>	Variable (0.3X SYBR)	N/A
25 mM MgCl <sub>2</sub> <sup>b</sup>	2 mM (1.5 – 4 mM)	2 mM (1.5 – 6 mM)
10 µM Primers (Forward and Reverse) <sup>a</sup>	0.2 µM (0.2 – 0.6 µM)	
StellarTaq DNA Polymerase (5 U/µL) <sup>c</sup>	0.02 U/µL (0.012 – 0.05 U/µL)	
dNTPs (10 mM each) <sup>a</sup>	0.2 mM	
Template DNAd	Variable	
PCR-grade water <sup>a</sup>	Up to final reaction volume	

### 2. Recommended reaction setup for intercalating dye-based one-step RT-qPCR and endpoint RT-PCR

Component	Final concentration	
	Intercalating dye-based RT-qPCR	Endpoint RT-PCR
10X Stellar Reaction Buffer A or B	1X	
Intercalating dye (e.g., 500X SYBR) <sup>a</sup>	Variable (0.3X SYBR)	N/A
25 mM MgCl <sub>2</sub> <sup>b</sup>	2 mM (1.5 – 4 mM)	2 mM (1.5 – 6 mM)
10 µM Primers (Forward and Reverse) <sup>a</sup>	0.2 µM (0.2 – 0.6 µM)	
StellarTaq DNA Polymerase (5 U/µL) <sup>c</sup>	0.02 U/µL (0.012 – 0.05 U/µL)	
StellarScript HT+ Reverse Transcriptase (200 U/µL) <sup>e</sup>	0.4 U/µL (0.4 – 3.6 U/µL)	
dNTPs (10 mM each) <sup>a</sup>	0.2 mM	
Template DNAd	Variable	
RNase Inhibitor (40 U/µL) (Optional) <sup>f</sup>	1 U/µL	
PCR-grade water <sup>a</sup>	Up to final reaction volume	

<sup>a</sup>Not supplied in the Watchmaker Stellar qPCR or RT-qPCR Kits.

<sup>b</sup>The MgCl<sub>2</sub> concentration may need to be optimized.

<sup>c</sup>Use 0.02 U/µL as an initial StellarTaq HS enzyme concentration for intercalating dye-based (RT)-qPCR or endpoint (RT)-PCR. Use lower enzyme concentrations, down to 0.012 U/µL if non-specific amplification is observed. Increase enzyme concentration, up to 0.096 U/µL, if PCR inhibition is observed.

<sup>d</sup>Template DNA/RNA input is assay-dependent and must be optimized.

<sup>e</sup>0.4 U/µL of StellarScript HT+ is a good starting concentration. Use lower concentrations, down to 0.4 U/µL, for reactions that do not contain inhibitors. Use higher concentrations, up to 3.6 U/µL for reactions that contain inhibitors.

<sup>f</sup>Watchmaker Genomics RNase Inhibitor (40 U/µL) is not included in the qPCR and RT-qPCR Kits but is supplied separately by Watchmaker Genomics. RNase Inhibitor can be included to prevent RNA degradation in samples containing RNases.

## Appendix B – Troubleshooting Guide

Challenge	Factors to consider	Guidance
PCR inhibition by inhibitors in sample type	StellarTaq HS concentration	Increase StellarTaq HS concentration (up to 0.096 U/μL)
	Additives	Add 1M betaine* and/or 0.2 mg/mL Bovine Serum Albumin* (BSA) to the reaction mix to increase inhibitor tolerance
Non-specific amplification	StellarTaq HS concentration	Reduce StellarTaq HS concentration (down to 0.012 U/μL)
	StellarScript HT+ concentration	Reduce StellarScript HT+ concentration (down to 0.4 U/μL)
	Cycling conditions	Reduce combined annealing and extension time
Fast PCR or RT-PCR (<10 minutes to <2 minutes for PCR or RT-PCR)	Buffer	Test 10X Stellar Reaction Buffer B (preferred buffer for fast PCR)
	StellarTaq HS concentration	Increase StellarTaq HS concentrations 10- to 20-fold higher than standard reactions <sup>8</sup>
	Primer concentrations	Increase primer concentrations 10- to 20-fold higher than standard reactions <sup>8</sup>
	StellarScript HT+ concentration	Increase StellarScript HT+ concentration (up to 6.4 U/μL)
High GC containing target or high secondary structures	Additive	Add 1M betaine* as an isostabilizing agent to the PCR
	Cycling conditions	Optimize the combined annealing/extension temperature across a range of 55°C to 72°C
Lower final fluorescence	Primer ratios	Optimize forward and reverse primer ratios
	StellarScript HT+ concentration	Reduce StellarScript HT+ concentration (down to 0.4 U/μL)
	StellarTaq HS concentration	Reduce StellarTaq HS concentration (down to 0.012 U/μL)
Mismatch extension discrimination (genotyping)	StellarTaq HS concentration	Reduce StellarTaq HS concentration (down to 0.012 U/μL)
	Buffer	Use 10X Stellar Reaction Buffer A
	Additive	Add 1M betaine* as an isostabilizing agent to the PCR

\*Not supplied in the Watchmaker Stellar qPCR or RT-qPCR Kits.

### References

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### Revision History

Version	Description	Date
1.0	• First release	03/2026



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