

# STELLARSCRIPT HT+ REVERSE TRANSCRIPTASE



**StellarScript® HT+ Reverse Transcriptase** is an engineered M-MLV variant with reduced RNase H activity and enhanced processivity, activity, thermostability, and inhibitor tolerance in comparison to wild-type enzyme.

#### HIGHLIGHTS

- Best-in-class thermostability and inhibitor tolerance enables a breadth of challenging applications, including RT-qPCR assays for pathogen detection
- · Custom formats, including high concentration, support lyophilization applications
- · Inhibitor tolerance ensures compatibility with a broad range of clinical samples
- Increased activity generates higher cDNA yields of longer lengths for high-sensitivity performance with degraded RNA and **inputs as low as 10 pg**

#### ADVANTAGES OF PARTNERING WITH WATCHMAKER

- Purpose-designed enzymes deliver outstanding performance from prototype to large volume production and across lots
- · Expedited custom labeling and kitting formats from bulk to finished goods
- Application-relevant kit-based lot testing
- IS013485-compliant Quality Management System
- · Flexible terms designed with both start-up and large organizational needs in mind

#### **APPLICATIONS**

- RT-qPCR/qRT-PCR
- Pathogen detection
- RT-PCR
- · First strand cDNA synthesis
- Primer extension

# INTRODUCING THE STELLARSCRIPT PORTFOLIO

StellarScript HT+ is a standout enzyme in a suite of tailored reverse transcriptase (RT) variants. These engineered RTs have a wide range of enhanced properties that fuel multiple applications. The portfolio includes:

- StellarScript for template switching and robust performance
- StellarScript HT for increased thermostability and inhibitor tolerance
- StellarScript HT+ for best-in-class thermostability and inhibitor tolerance to enable challenging applications

#### Table 1. Overview of StellarScript portfolio enzyme properties

		StellarScript	StellarScript HT	StellarScript HT+
Property	Optimal Temp.	42°C	42°C – 50°C	42°C – 65°C
	Thermostability	+	++	+++
	Inhibitor Tolerance	+	++	+++
	Template Switching	+	-	+

"+" signs indicate strength of a property for a specific enzyme.

### SAFEGUARD PERFORMANCE WITH INHIBITOR TOLERANCE

Inhibitors can dampen reverse transcription efficiency and impact sensitivity in applications such as RT-qPCR. Improved robustness in the presence of common inhibitors safeguards performance and ensures compatibility across a wide range of sample types and sources. StellarScript HT+ delivers the highest level of inhibitor tolerance across the StellarScript portfolio.







FIGURE 1. Robust performance in the presence of common inhibitors. StellarScript, StellarScript HT, and StellarScript HT+ were run in oligo-dT-primed first strand synthesis for 30 min using 10 ng total liver RNA as template in varying concentrations of common inhibitors. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of  $\beta$ -actin gene to generate a 90 bp amplicon.  $\Delta$ Cq values were calculated: Cq(with inhibitor) - Cq(without inhibitor). Lower  $\Delta$ Cq values indicate increased inhibitor tolerance.

#### STELLARSCRIPT HT+ FOR MAXIMUM THERMOSTABILITY

Increased thermostability enables reverse transcription at elevated temperatures to overcome RNA template secondary structure and generate cDNAs from difficult targets, such as viral RNA. It further improves specificity by minimizing nonspecific primer binding.

StellarScript HT+ is the most thermostable enzyme in the portfolio, generating higher cDNA yields and better maintaining its folded protein structure at elevated temperatures relative to SuperScript IV and Maxima H Minus.



FIGURE 2. StellarScript HT+ provides equivalent processivity at elevated temperatures. Watchmaker's StellarScript HT+ and ThermoFisher Scientific's SuperScript IV and Maxima H Minus were run in an oligo-dT-primed first strand synthesis at 50°C, 55°C, 60°C, or 65°C for 30 min using a 0.5 to 9 kb RNA ladder as template. All enzymes have robust processivity up to 60°C.



FIGURE 3. StellarScript HT+ delivers superior thermostability. (A) Watchmaker's StellarScript HT+ and ThermoFisher Scientific's SuperScript IV and Maxima H Minus were run in an oligo-dT-primed first strand synthesis was employed for 25 min using 10 ng total liver RNA, as indicated. Resulting cDNA mass was assessed via qPCR using primers targeting the 5' end of  $\beta$ -actin gene to generate a 90 bp amplicon. StellarScript HT produced higher yields (indicated by lower Cq values) than SuperScript IV and Maxima H Minus at any evaluated temperature, indicating improved efficiency at elevated temperatures. (B) Enzymes were further assessed via static light scattering under increasing temperature to determine their respective aggregation temperatures, at which point protein unfolding begins and (C) during a time course study at 60°C to measure their stability in reverse transcription reaction conditions. The enhanced thermostability of StellarScript HT+ is demonstrated by its increased aggregation temperature and improved stability over time at 60°C.





## OUR TECHNOLOGY: THE NEW FRONTIER OF PROTEIN ENGINEERING

Industry-leading assays require best-in-class reagents.

Watchmaker's focus on application-driven development and deep domain expertise in advanced enzyme engineering, computational biology, and high-stringency protein production enables us to address unmet needs with respect to performance, scalability, and consistency of supply.

Our purpose-designed enzymes deliver outstanding performance from prototype to large volume production.



### STELLARSCRIPT HT+ SPECIFICATIONS

- Concentration: 200 U/µL
- Purity (SDS-PAGE): >99%
- dsDNA exonuclease: <1% released\*</li>
- SSDNA exonuclease: <1% released\*</li>
- DNA contamination (E. coli, mammalian, library): <10 copies\*
- Phosphatase assay: <1% release\*</li>
- Endonuclease: Not detectable\*
- Nonspecific RNase: Not detectable\*

\*As assessed using 400 U of enzyme input per assay.

# ALSO AVAILABLE:

# High-purity RNase Inhibitor

Protect what's precious for your single cell, single nuclei, and RT-qPCR assays.

# StellarTaq<sup>™</sup> DNA Polymerase

Combat inhibitors with a robust and fast polymerase – excellent for qPCR applications.



Contact <u>sales@watchmakergenomics.com</u> or visit <u>watchmakergenomics.com/stellarscript-htplus</u> to learn more.

PRODUCT	10 kU <sup>1</sup>	40 kU <sup>1</sup>	200 kU <sup>1</sup>
<b>StellarScript Multi Reverse Transcriptase Sample Pack (200 U/μL)</b> incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT, and 50 μL each of StellarScript RT, StellarScript HT RT, and StellarScript HT+ RT	7K0083-50UL	-	-
StellarScript HT+ Reverse Transcriptase Kit (200 U/µL) incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT	7K0081-50UL	7K0081-200UL	7K0081-1ML

<sup>1</sup>One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in 10 min at 37°C using poly(A)/oligo (dT) as a substrate.

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