HighTherm™ Reverse Transcriptase

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-1991	10,000u (200u/µl)	HighTherm™ RTase - 2 x 25µl	5x HighTherm Buffer – 1 x 200µl
AZ-1994	40,000u (200u/µl)	HighTherm™ RTase - 2 x 100µl	5x HighTherm Buffer – 4 x 200µl

Description

HighTherm™ Reverse Transcriptase is an engineered mutant of MMLV providing increased specificity and exceptionally high cDNA yields even with complex RNA templates such as viral targets. This novel enzyme is fully functional across a wide temperature range of 38°C to 55°C which enables excellent assay flexibility and high temperature cDNA synthesis for complex RNA secondary structures. HighTherm™ Reverse Transcriptase is blended with a potent RNase inhibitor preventing degradation of RNA by contaminating RNase. The 5x Buffer contains optimal levels of dNTPs, $MgCl_2$ and enhancers.

- More representative full-length cDNA due to reduced RNase H activity.
- HighTherm™ Reverse Transcriptase coupled with an optimized 5x Buffer system generates consistent, high-yield cDNA.
- Optimized for dilute and low-copy input RNA.

Storage

HighTherm™ Reverse Transcriptase is shipped on blue or dry ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided. When stored as specified, the kit is stable for 12 months from date of receipt.

Important Guidelines

5x HighTherm Buffer: The 5x Buffer contains optimal levels of dNTPs (5mM), 15mM $MgCl_2$, enhancers, and stabilizers. The buffer has been designed to deliver maximum efficiency and very high-quality full-length cDNA. We do not suggest the use of additional reaction components.

Template: Ideally, we suggest the use of 4.0pg to 0.5µg Total RNA or oligo(dT) purified mRNA to maintain accurate relative cDNA representation. HighTherm™ reverse transcriptase exhibits greater activity than many competing enzymes and generates very high cDNA yields, from small amounts of RNA.

Primers: We recommend the use of random hexamers (concentration of 2 - 5µM), Oligo-dT_{18} primers (concentration of 1µM) or gene-specific primers (concentration of 1pM). For unbiased cDNA synthesis for subsequent qPCR, we suggest a combination of random hexamers and oligo-dT primers.

Incubation temperature: We recommend a temperature of 42°C for 30 minutes for the vast majority of applications (<65% GC content). Incubation temperatures of up to 55°C may be used for regions containing significant secondary structure (>65% GC content).

qPCR reaction set-up: We recommend 4.0µl of the cDNA produced per 20µl real-time PCR reaction. Alternatively, cDNA may be stored at 4°C for 1 week or -20°C for long term storage.

Reaction setup

1. Prepare a master mix based on following table (and allow 5x HighTherm™ Buffer to thaw):

Component	20µl Reaction	Final Concentration/Notes
5x HighTherm™ Buffer	4 µl	1x
HighTherm™ RTase	1.0 µl	Add prior to RNA template
Total RNA (4pg – 0.5µg)	variable	Total RNA or mRNA
Primers (see Guidelines)	variable	
PCR-grade water	Up to 20 µl final volume	

* For alternative total reaction volumes (eg. 25 µl), scale all components proportionally to maintain final concentrations.

2. Incubation and subsequent enzyme denaturation:

	Temperature & Time	Notes
Incubation	42°C, 30 minutes	For RNA with high degree of secondary structure, incubate at 55°C
Denaturation	85°C, 10 minutes	This will denature RTase

Quality Control

HighTherm™ Reverse Transcriptase is tested extensively for reproducibility, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. HighTherm™ Reverse Transcriptase is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

Limitations of Use

This product is intended for research purposes only and is not intended for any animal or human therapeutic use.

Technical Support

For Trouble-shooting and Technical Guidance, please contact us at tech@azuragenomics.com and provide reaction conditions including incubation temperature and time, and RNA concentration.