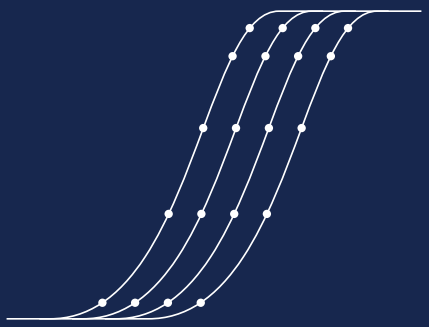

# Gene Expression & qPCR

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# HighTherm™ Reverse Transcriptase

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 efficiently synthesizes a complementary DNA strand from ssRNA, ssDNA or RNA:DNA hybrids with significantly reduced Ribonuclease H activity. HighTherm™ RT generates cDNA from 100bp to > 12Kb.

## Applications

- 1-Step and 2-Step RT-qPCR
- End-point RT-PCR
- Array labelling
- cDNA libraries
- 3' and 5' RACE

## Yield

- More representative full-length cDNA due to reduced RNase H activity (100bp to > 12Kb)

## Sensitivity

- Reproducible cDNA synthesis from a wide range of template (1pg to 5µg of total RNA template)

## Thermostability

- Retains greater than 90% of enzymatic activity following 55°C incubation for 60 minutes.

HighTherm™ Reverse Transcriptase

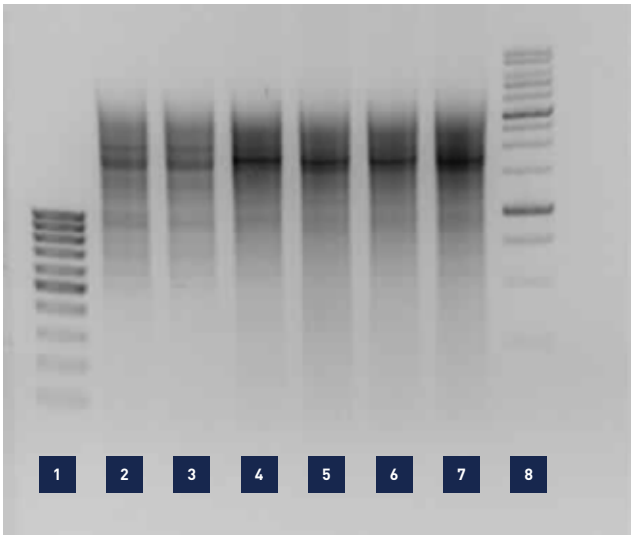


Fig 1.

- cDNA libraries from total human nervous tissue RNA synthesized by HighTherm™ Reverse Transcriptase and Competitor I.
- Reaction mix (10 µl) contained 200u HighTherm™ and 0.5 µg total RNA. Reactions were carried out at 42°C. The aliquots were then amplified by Taq polymerase in standard PCR.
- Lane 1 : 100 bp Ladder
- Lanes 2 and 3 : SuperScript® III Invitrogen
- Lanes 4 - 7 : HighTherm™
- Lane 8 : 1000 bp ladder, reference bands at 1000 and 3000 bp

HighTherm™ Reverse Transcriptase exhibits greater sensitivity in cDNA library construction.

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# HighTherm™ Reverse Transcriptase

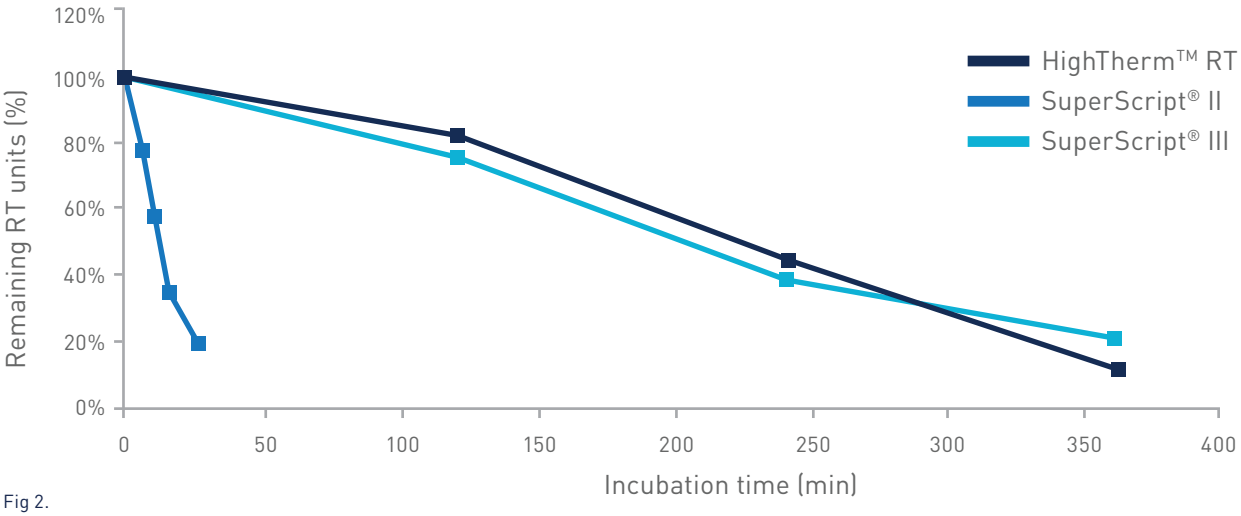
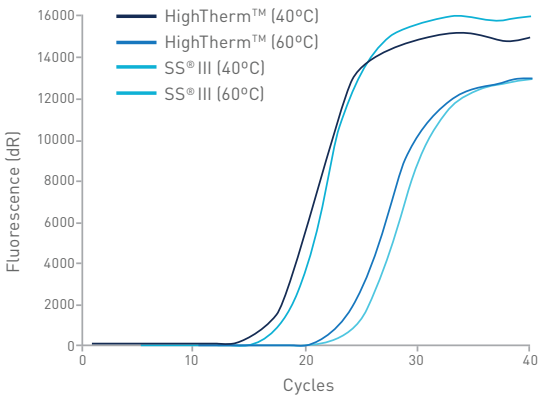



Fig 2.

Thermostability analysis at 55 °C in which HighTherm™ Reverse Transcriptase retains enzymatic activity during high temperature incubation.




## HighTherm™ qPCR

Fig 3.

- Real-Time PCR analysis of cDNA generated by HighTherm™ Reverse Transcriptase and SuperScript® III (InVitrogen): cDNA Synthesis reactions contained 1µl (200 u/µl) reverse transcriptase and 0.5 µg total RNA.
- Independent reactions were carried out at 40°and 60°C followed by qPCR in a SYBR Green assay (measuring housekeeping gene GAPDH).
- HighTherm™ Reverse Transcriptase exhibits robust activity (early Ct) and a wide thermostability range.

| Product | Pack Size | Catalog No. | Price |
|---|---|---|---|
| HighTherm™ Reverse Transcriptase | 10,000u (200u/µl) | AZ-1991 | $165 |
| HighTherm™ Reverse Transcriptase | 40,000u (200u/µl) | AZ-1994 | $575 |