ThermaStop-RT™

- can be used in either one-step or two-step RT-PCR containing gene-specific primers.
- interacts with the reverse transcriptase at low temperatures to reduce priming errors that lead to non-specific products.
- improves detection sensitivity and specific product yield.
- One unit of ThermaStop-RT is the amount required for optimal results for RT-PCR containing 50 units of reverse transcriptase and 1 unit of hot-start Taq DNA polymerase in a volume of 20 µl.

PREPARATION AND STORAGE:

To prepare a 5 Units/µl ThermaStop-RT stock from 125 Units of dry reagent:

- Centrifuge tube briefly to insure the dried reagent is at the bottom of the tube.
- Add sterile, molecular-grade 10 mM Tris-Cl, pH 8.3; 25 µl for tubes containing 125 units, or 125 µl for vials containing 625 units.
- Vortex tube for at least 1 minutes, then centrifuge briefly.
- Aliquot into smaller volumes, if desired.
- If frozen, ThermaStop-RT should be divided into aliquots to limit freeze-thaw to a maximum of five times.

USE:

- Two step RT-PCR samples should contain 1 unit of ThermaStop-RT with 50 units MMLV-derived reverse transcriptase (e.g. SuperScript III from Fisher Scientific, or PrimeScript from Takara) in a 20 microliter volume. Following reverse transcription, heat inactivate the reverse transcriptase and dilute samples into an appropriate PCR buffer containing a hot-start Taq polymerase.
- One-step RT-PCR samples should contain 1 unit of ThermaStop-RT with 50 units reverse transcriptase and 1 unit hot-start Taq polymerase in 20 microliters.
- Higher concentrations of ThermaStop-RT may be necessary for samples containing higher concentrations of enzymes.
- A 15 to 30 minute reverse transcription step at 50^{o}C or higher is recommended. Temperatures below 45^{o}C are not recommended, as cDNA synthesis will be lower.

NOTE:

- Use with random primers or oligo dT is not recommended.
- ThermaStop-RT should not be used in combination with ThermaStop™ or ThermaGo™.
- Antibody-based hot start Taq or heat-activated Taq are recommended.

- PCR annealing temperature should be at least 60^{o}C.
- ThermaStop-RT™ has been tested using MMLV-derived reverse transcriptase and Taq polymerase. Use with other enzymes may be possible.