Therma*Stop-RT*™
Instructions for Use.

- 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 Therma*Stop-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:

- Therma*Stop-RT* is provided as a 5 Unit per microliter solution and may be stored at 4°C or frozen at -20°C in dark (or light protected) tubes.
- If frozen, Therma*Stop-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 Therma*Stop-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 Therma*Stop-RT* with 50 units reverse transcriptase and 1 unit hot-start Taq polymerase in 20 microliters.
- Higher concentrations of Therma*Stop-RT* may be necessary for samples containing higher concentrations of enzymes.
- A 15 to 30 minute reverse transcription step at 50°C is recommended. Temperatures below 45°C are not recommended, as cDNA synthesis will be lower.

NOTE:

- Use with random primers or oligo dT is not recommended.
- Therma*Stop-RT* should not be used in combination with Therma*Stop-RT* or ThermaGo.
- Antibody-based hot start Taq or heat-activated Taq are recommended.
- PCR annealing temperature should be at least 60°C.
- Therma*Stop-RT* has been tested using MMLV-derived reverse transcriptase and Taq polymerase. Use with other enzymes may be possible.
- Increasing Therma*Stop-RT* to 2 units per sample or reducing reverse transcriptase concentration may improve results for some RT-PCR assays.