Product Bulletin

ThermaStop™

Innovative Hot Start and Cold Stop Reagent for Enhanced Taq DNA Polymerase Specificity and Product Yield

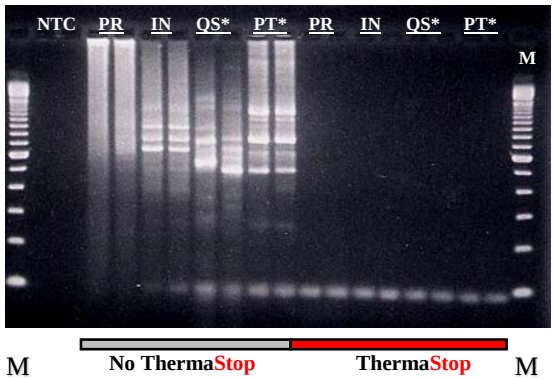

Figure 1 - Universal Hot Start Reagent: ThermaStop improves product specificity and yield of commercial non-hot start and hot start Taq DNA polymerases *represents hot-start enzyme, M is 100 bp ladder.

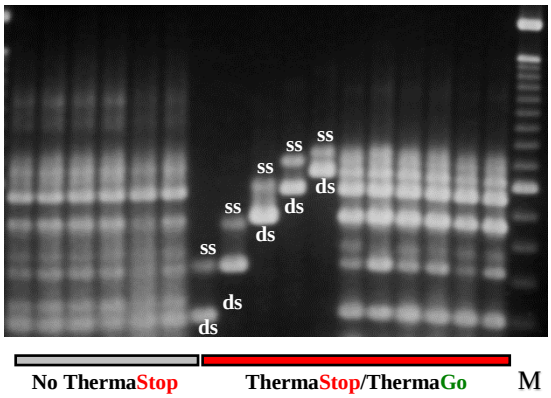

Figure 2 – I Multiplexing Without Extensive Primer Optimization: Five pairs of asymmetric PCR primers not optimized for compatibility *in silico* were tested either without or with ThermaStop/ThermaGo (TS/TG). Individual pairs were also amplified with TS/TG. Reactions with TS/TG gave cleaner mixtures of the expected double-stranded and single-stranded amplicons with less nonspecific products. ds, double-stranded DNA; ss, single-stranded DNA; M, 100 bp ladder

ThermaStop™ is a reversible hot start reagent that is compatible with all Taq DNA polymerases. It acts directly on the polymerase to prevent non-specific enzymatic activity below 50°C. Polymerase activity is fully restored at 60°C, but is inhibited again by ThermaStop by cooling the reaction.

ThermaStop Out-Performs Hot Start Antibodies:

1. Effectively suppresses formation of primer dimers/non-specific products before PCR - eliminates amplification in no template controls (Figs. 1 and 2).
2. Stabilizes master mixes for extended times - allows convenient preparation and storage of master mixes at room temperature prior to PCR for up to 36 hours (Fig 3).
3. Rapidly inactivated in the first PCR cycle - no need for extended high temperature incubation.
4. Reversible, immediately re-activated upon cooling - enables resumption of PCR cycling; safeguards product integrity at endpoint for downstream applications, including Next-Gen sequencing and nested-PCR (Fig. 4).
5. Stable non-aptamer, chemically modified nucleic acid not affected by proteases.

How To Use:

One unit of ThermaStop is defined as the amount required for maximum hot start activity in amplification reactions containing 1 unit of Taq DNA polymerase in a volume of 25 µl. ThermaStop does not contain magnesium, dNTPs, or PCR buffer components.

To use, add an equal number of units of ThermaStop and Taq DNA polymerase to PCR master mix. **Note:** PCR annealing temperature should be 60°C or above to insure full polymerase activity. ThermaStop in combination with ThermaGo™ prevent mispriming before, during, and after PCR.

Typical 25 ul PCR

Reagent	Final Concentration	Volume
10X PCR Buffer	1X	2.5 µl
2 µM primers	0.2 µM	2.5 µl
5 U/µl Taq	0.05 U/µl	0.25 µl
5 U/µl ThermaStop	0.05 U/µl	0.25 µl
Template		x µl
H_2O		QS 25 µl

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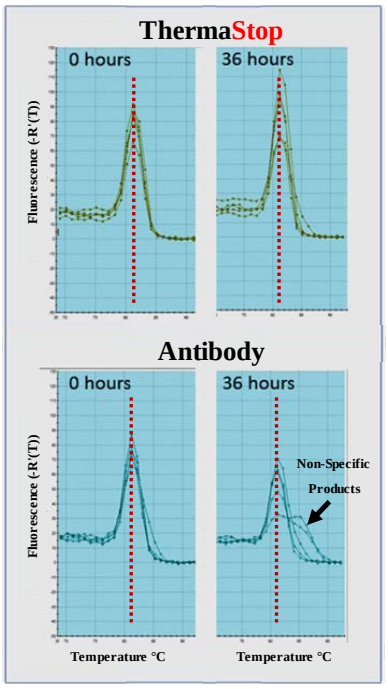

Figure 3 – Extended Hot Start Capabilities of ThermaStop Compared to an Antibody: Melt analysis of a complete master mix including DNA at 25°C incubated for increasing periods of time prior to PCR.

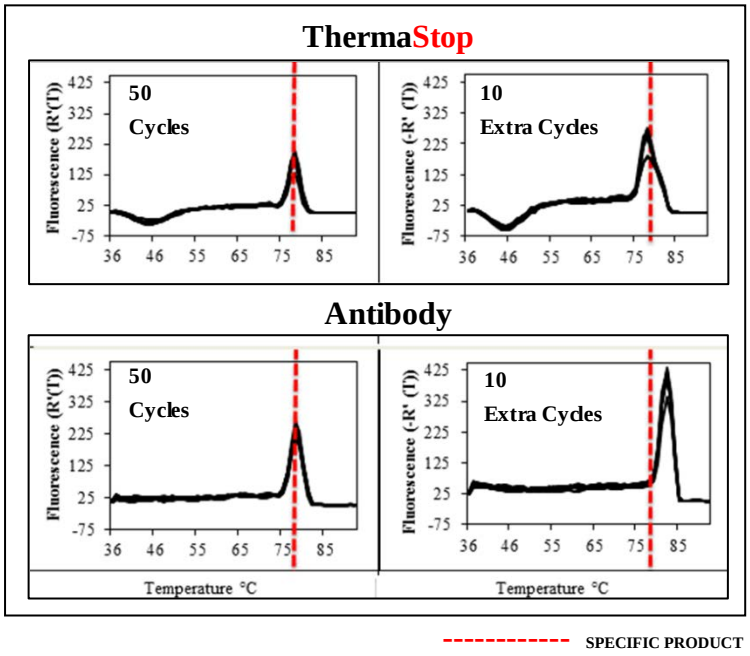

Figure 4 – ThermaStop enables cold stop AFTER PCR: PCR can be stopped and resumed without altering amplification products. ThermaStop safeguards product integrity for downstream applications (next generation sequencing, nested-PCR).

Applications of Interest:

Hot-start protection for all forms of PCR. Enhanced detection of low-copy number targets (single-cell PCR, digital PCR, rare target detection in liquid biopies/tumor samples). Enhances accuracy of highly multiplexed-PCR for targeted next-generation sequencing and other applications.

How to Prepare for Use:

ThermaStop is shipped as a dry reagent in 500 or 2500 units. To prepare 5 Units/µl ThermaStop, add 100 µl molecular-grade 10 mM Tris-Cl, pH 8.3 to 500 units dry reagent (500 µl 10 mM Tris-Cl, pH 8.3 to 2500 units dry reagent), vortex 1-2 minutes, then centrifuge briefly. Allow tube to sit at room temperature for 15 minutes with occasional mixing to ensure reagent is completely dissolved.

Recommended Storage:

Store ThermaStop at 4˚C or -20˚C in the dark or in light protected tubes. If frozen, divide stock into small volume aliquots to avoid freezing and thawing more than 5 times.

Expected Results:

ThermaStop will reduce the production of non-specific products and generate higher yields of the correct products (e.g. stronger probe signals, correct bands on gels) compared to non-hot start conditions. Please contact ThermaGenix Technical Support if you have further questions.

Ordering Information

Part Number	Description	Number of Taq DNA Polymerase Equivalent Units
TS-500	ThermaStop- 500 Units	500
TS-2500	ThermaStop- 2500 Units	2500

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