Effective Hot Start PCR

TaqStart® Antibody for fast, convenient hot start

- **Convenient, room temperature reaction set-up**
- **Increased reaction specificity**
- **Use TaqStart with any full-length *Taq* polymerase**
- **Also available in bulk quantities**

The polymerase chain reaction (PCR) is a versatile technique for exponentially amplifying small amounts of target DNA. However, it often results in the amplification of non-target sequences that are also present in the reaction mix. Such "background" products are rarely beneficial, and in large amounts can decrease the yield of the desired product and needlessly complicate its analysis. Several methods, collectively referred to as "hot start PCR", have been developed to limit the generation of background products, and provide higher reaction specificity and yield.

Nonspecific Amplification

PCR is a very sensitive technique; however, prior to thermal cycling (i.e., during reaction set up), PCR reactions are susceptible to the nonspecific binding of primers to template DNA, contaminant DNA, or even other primers. At suboptimal temperatures, low level polymerase activity results in the extension of these misprimed sequences, and ultimately leads to the generation of nonspecific products and primer artifacts (e.g., primer-dimers). Amplification of nonspecific background products reduces the yield of target DNA, as nucleotides and primers that would have been used to amplify the target sequence get used up in side reactions. In addition, the presence of background products hinders the isolation and subsequent analysis of the target product.

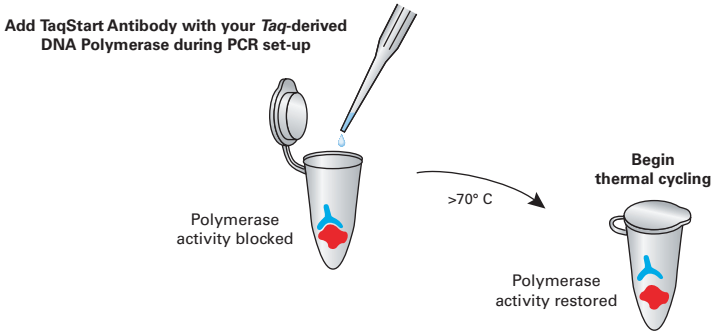

Figure 1. TaqStart Antibody inhibits polymerase activity before thermal cycling begins.

Hot Start PCR Methods

Hot-start PCR methods reduce the generation of nonspecific products and primer artifacts. A variety of hot start methods exist (1), and although the specifics of each vary, most function by restricting the availability of an essential reaction component until the reaction temperature is high enough to prevent nonspecific priming. For example:

Physical removal of essential reaction components: The earliest attempts at hot start PCR simply delayed the addition of the polymerase until the reaction temperature reached 94°C, when the DNA is fully denatured. Although this method works, it requires extra handling steps, which are inconvenient when performing multiple reactions and increase the likelihood of sample contamination.

Sequestration of components within the reaction: Most of the methods in this group use heat sensitive materials, such as wax or agarose beads, to separate an essential component (e.g., *Taq* DNA polymerase or $MgCl_2$) from the rest of the reaction mix. Once the reaction temperature is high enough to melt the wax or agarose, the sequestered component is released into the reaction mix, allowing PCR to proceed. Another method of sequestration involves the formation of a magnesium precipitate. The precipitate sequesters magnesium ions (which are required by the polymerase) from the reaction mix, and releases them when the reaction temperature is between 50–95°C (2).

Chemical modification of the polymerase: In this hot start method, the polymerase is reversibly inactivated by chemical modification. The enzyme is reactivated by preheating the reaction mix at 94–95°C for 9–12 minutes. This can be problematic, as high temperature reactivation can result in depurination of the DNA template (3), reducing the quality of the products produced. In addition, the long reactivation time increases the length of the PCR reaction and lowers throughput.

Reversible, ligand-mediated polymerase inhibition: The most promising hot start methods use an oligonucleotide or antibody ligand to reversibly inhibit the polymerase. Ligand-mediated inhibition is superior to inactivation of the polymerase by chemical modification because there is no need for a lengthy, high temperature reactivation step. Enzyme activity is restored as the ligand simply dissociates or denatures at higher temperatures. In addition, once inhibition has been eliminated, the polymerase retains most, if not all, of its original activity.

Effective Hot Start PCR...continued

Each of the ligand-mediated inhibition methods differs as a result of the unique properties of the ligand involved. During antibody-mediated hot start, the polymerase is inhibited until the antibody is denatured by the high temperatures in the first reaction cycle. Because the antibody is completely denatured during this cycle, it no longer has any effect on the polymerase, regardless of the reaction temperature.

TaqStart Antibody

In order to provide our customers with the benefits of automatic hot start PCR, most of our PCR enzyme systems are blended with **TaqStart Antibody.** TaqStart is an anti-*Taq* monoclonal antibody that inhibits the enzymatic activity of *Taq* and *Taq*-derived DNA polymerases during room temperature reaction assembly (Figure 1). Full polymerase activity is restored when the antibody is denatured during the first PCR cycle, allowing amplification to proceed normally. Inclusion of TaqStart Antibody significantly improves PCR efficiency and specificity by reducing or eliminating nonspecific amplification and the formation of primer-dimers and other artifacts prior to thermal cycling (4). In addition to our enzyme blends, we also offer TaqStart Antibody as a stand-alone product, which allows customers to use it with the *Taq*-derived DNA polymerase of their choice (i.e., native proteins, recombinant proteins, and N-terminal deletion mutants).

Greater Specificity and Yield

To demonstrate TaqStart's ability to effectively inhibit *Taq*-derived polymerase activity, isothermal extension reactions were performed on single-stranded ϕX174 viral DNA using **TITANIUM™ *Taq* DNA Polymerase** with and without TaqStart Antibody (Figure 2, Panel A). Without TaqStart, TITANIUM *Taq* was able to synthesize over 100 ng of DNA (red line), whereas the addition of TaqStart clearly inhibited DNA synthesis (pink line).

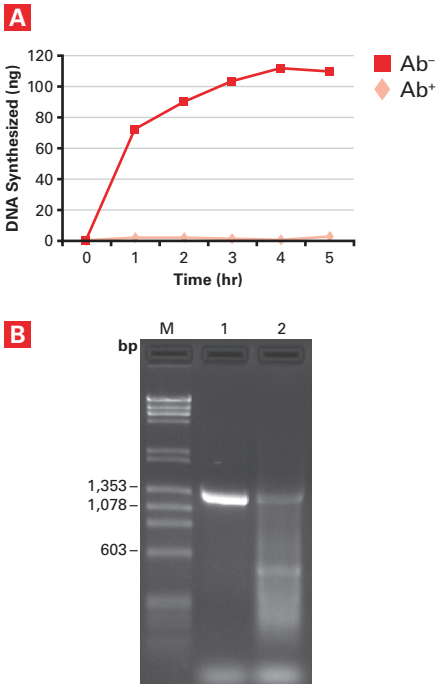

Figure 2. TaqStart Antibody provides automatic hot start for increased enzyme specificity and product yield. Panel A. Isothermal extension reactions were performed at 37°C for 5 hr, using single-stranded ϕX174 viral DNA and TITANIUM *Taq* DNA Polymerase plus (+) or minus (–) TaqStart Antibody. TaqStart clearly inhibited DNA synthesis (pink line). **Panel B.** A 1.3 kb portion of the human transferrin receptor gene was amplified from a mixture of human placenta genomic DNA and QUICK-Clone™ cDNA. Reactions were performed using TITANIUM *Taq* with TaqStart Antibody (Lane 1) or without TaqStart Antibody (Lane 2). As seen in Lane 1, TaqStart greatly enhanced enzyme specificity.

TaqStart's ability to inhibit nonspecific amplification can also be seen in PCR reactions (Figure 2, Panel B) in which a 1.3 kb portion of the human transferrin receptor gene was amplified from a mixture of human placenta genomic DNA and QUICK-Clone™ cDNA. Reactions were performed with TITANIUM *Taq* plus TaqStart Antibody (Lane 1) or TITANIUM *Taq* alone (Lane 2). The reaction containing TaqStart Antibody (Lane 1) resulted in far fewer background products than the reaction lacking the antibody (Lane 2), showing that TaqStart greatly enhanced enzyme specificity and product yield.

Product	Size	Cat. No.
TaqStart Antibody		
	200 rxns	639250
	500 rxns	639251

Components

- TaqStart® Antibody
- Dilution Buffer

Related Products

- TITANIUM™ *Taq* DNA Polymerase (Cat Nos. 639208, 639209, 639242, 639210 & 639211)
- Human Placenta QUICK-Clone™ cDNA (Cat No. 637208)

Notice to Purchaser

Please see the Hot Start Antibody licensing statement on page 25.

With TaqStart Antibody, you get effective hot start without special heating cycles, wax, or expensive enzymes. TaqStart significantly improves PCR specificity and product yield by reducing or eliminating nonspecific products and primer artifacts.

References

1. Martel, M. *et al.* (2007) In *PCR: Methods Express,* Eds. Hughes S. & Moody, A. (Scion Publishing Ltd., Oxfordshire), pp 24–25.
2. Barnes, W. M. & Rowlyk, K. R. (2002) U.S. Patent No. 6403341, Magnesium precipitate hot start method for PCR.
3. Lindahl, T. & Nyberg, B. (1972) *Biochemistry* **11**(19):3610–3618.
4. Kellogg, D. E. *et al.* (1994) *BioTechniques* **16**(6):1134–1137.