



How is Hot-Start Technology Beneficial For Your PCR?



Nonspecific amplification is one of the major issues that can drastically impact PCR performance, resulting in one or more of: low yield of target amplicons, reduced sensitivity in detection of target amplicons, unreliable results for interpretation, and poor efficacy in downstream applications.

A common source of nonspecific amplification is the extension of misprimed sequences by DNA polymerases and the formation of primer-dimers. One workaround to help avoid nonspecific amplification is to prepare the PCR reaction mixture on ice. The colder temperature helps lower the activity of the DNA polymerase; however synthesis of undesirable products may still occur before the start of PCR. Another solution is to use a hot-start DNA polymerase. Hot-start modifications inhibit DNA polymerase's activity at room temperature, preventing spurious bands from nonspecific amplification.

What are the benefits of hot-start technology?

- Prevents extension of primers binding to template sequences with low homology (mispriming)
- Prevents extension of primers binding to each other (primer-dimer formation) during reaction setup
- Increases sensitivity and yield of the desired target fragments
- Enables PCR setup on high-throughput or automated liquid-handling platforms as reactions are stable at room temperature without compromising specificity

How does hot-start technology work?

Methods of hot-start PCR employ an enzyme modifier such as a chemical group, antibody, Affibody molecule, or aptamer. Two of the most common methods used are chemical modification and antibodies. While they all inhibit polymerase activity at room temperature, there are some key differences among them.

Hot-start technology	Benefits	Considerations
Chemical Polymerases are covalently linked with chemical groups to block enzyme activity at room temperature. Example: AmpliTaq Gold DNA Polymerase	• Generally more stringent than other hot-start methods • Gradual enzyme activation possible • Free of animal-origin components	• Longer activation time required for the polymerase to become fully active • Full activation of the enzyme often not possible • Can affect amplification of targets longer than 3 kb
Antibody Polymerases are bound by antibodies at their active sites to block enzyme activity at room temperature. Examples: DreamTaq Hot Start DNA Polymerase , Platinum II Taq DNA Polymerase , Platinum SuperFi II DNA Polymerase	• Enzyme features similar as the non-hot-start version since antibodies do not alter the polymerase • Short activation time as the initial denaturation step of PCR activates the polymerase • Full enzyme activity restored after activation	• Antibodies may be of animal origin • Higher level of exogenous proteins (i.e., antibodies) present in the reaction

Affibody molecule Polymerases are bound by affibody molecules (oligonucleotides) at their active sites to block enzyme activity at room temperature. Examples: Phire Hot Start II DNA Polymerase, Phusion Plus DNA Polymerase	• Less protein (compared to antibody) present in the reaction • Short activation time • Free of animal-origin components	• May be less stringent than the antibody-based method • Assembled reactions may not be stable at the benchtop for a long time
Aptamer Polymerases are bound by aptamers (oligonucleotides) at their active sites to block enzyme activity at room temperature.	• Short activation time • Free of animal-origin components	• May be less stringent and may result in nonspecific amplification • Assembled reactions may not be stable at the benchtop for a long time. • May not work well with primers of low melting temperatures (due to low activation temperature and reversible activation)



Resources

Find videos, webinars, articles, and tools in our [molecular biology resource library](#) ›

More spotlight articles

- [PCR infographic](#)
- [Selecting PCR enzymes](#)
- [The power of two](#)

Related products

- [Thermo Scientific DNA polymerases](#)
- [Applied Biosystems and Invitrogen DNA polymerases](#)
- [OEM & customized solutions](#)

For Research Use Only. Not for use in diagnostic procedures.