Finnzymes Reagents - Optimizing Tm and annealing

HOME

Important instructions on annealing temperature

We recommend calculating primer Tm with the modified Breslauer's method[1]. To determine the annealing temperature for the actual PCR run when using different Thermo Scientific Finnzymes DNA polymerases or kits, see the table below. A few notes about primer design are also included.

Phusion High-Fidelity DNA Polymerase

Primer conc. (nM)	Use the actual primer concentration in the calculation. Recommendation for Phusion DNA Polymerase is 500 nM, but it can be varied between 200 nM - 1000 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.
Notes	For primers max 20 nt use the lower Tm given by the calculator for annealing. For primers > 20 nt use an annealing temperature 3°C higher than the lower Tm given by the calculator. Example: Tm's given by the calculator are 66.5°C and 65.0°C => Use an annealing temperature of 68.0°C in the actual run. With Phusion Hot Start DNA Polymerase, use primers with Tm 60°C or higher. With Phusion Hot Start II DNA Polymerase and all non-hot start Phusion DNA Polymerases primers with lower Tm can also be used. With Phusion Flash DNA Polymerase and Phusion Blood DNA Polymerase, do not perform annealing below 50°C. If the amplification fails with the recommended annealing temperature, use a temperature gradient to optimize the annealing. The annealing gradient should range from the original annealing temperature to the extension temperature (two-step PCR). If high DMSO concentration is used, the annealing temperature determined by the guidelines above must be lowered, as DMSO decreases the melting point of the primers. It has been reported that 10% DMSO

	decreases the melting temperature by 5.5-6.0°C.[2]

Phire Hot Start DNA Polymerase

Primer conc. (nM)	Use the actual primer concentration in the calculation. Recommendation for Phire Hot Start DNA Polymerase is 500 nM, but it can be varied between 200 nM - 1000 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.
Notes	For primers max 20 nt use the lower Tm given by the calculator for annealing. For primers > 20 nt use an annealing temperature 3°C higher than the lower Tm given by the calculator. Example: Tm's given by the calculator are 66.5°C and 65.0°C => Use an annealing temperature of 68.0°C in the actual run. With Phire Hot Start DNA Polymerase, use primers with Tm 60°C or higher. With Phire Hot Start II DNA Polymerase primers with lower Tm can also be used. If the amplification fails with the recommended annealing temperature, use a temperature gradient to optimize the annealing. The annealing gradient should range from the original annealing temperature to the extension temperature (two-step PCR). If high DMSO concentration is used, the annealing temperature determined by the guidelines above must be lowered, as DMSO decreases the melting point of the primers. It has been reported that 10% DMSO decreases the melting temperature by 5.5-6.0°C.[2]

DyNAzyme I, DyNAzyme II and DyNAzyme EXT DNA Polymerases

Primer conc. (nM)	Use the actual primer concentration in the calculation. Recommendation for all DyNAzyme DNA Polymerases is 300 nM -1000 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.

Notes	In the actual PCR run use an annealing temperature 5°C lower than the lower Tm given by the calculator. Example: Tm's given by the calculator are 66.5°C and 65.0°C => Use an annealing temperature of 60.0°C in the actual run. If high DMSO concentration is used, the annealing temperature determined by the guidelines above must be lowered, as DMSO decreases the melting point of the primers. It has been reported that 10% DMSO decreases the melting temperature by 5.5-6.0°C.[2]

DyNAmo SYBR Green qPCR kits

Primer conc. (nM)	Use the actual primer concentration in the calculation. Recommendation for most DyNAmo SYBR Green qPCR Kits is 500 nM. For F-400S/L and F-400RS/L the recommendation is 300 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.
Notes	In the actual PCR run use an annealing temperature 5°C below the lower Tm calculated for the primers. Example: Tm's given by the calculator are 66.5°C and 65.0°C => Use an annealing temperature of 60.0°C in the actual run. With DyNAmo Flash SYBR Green qPCR Kit a combined annealing and extension step at 60°C works well for most amplicons, if primers are designed to anneal efficiently at 60°C (Tm of the primers is about 65°C). If the primers cannot be designed to anneal at 60°C, the annealing and extension steps can be performed separately. In that case use an annealing temperature 5°C below the lower Tm calculated for the primers.

DyNAmo Probe qPCR Kits

Primer conc. (nM)	Use the actual primer or probe concentration in the calculation. Recommendation for most chemistries with DyNAmo Probe qPCR Kits is between 50 - 1000 nM.
Salt (mM)	Always use the default 50 mM salt concentration in the calculation.
Notes	In the actual PCR run annealing temperature depends

on the chemistry used. With TaqMan chemistry primers and probes are usually designed to be annealed and extended at 60°C. Design primer Tm to be about 5 degrees above the annealing temperature and probe Tm about 10 degrees above the primer Tm. For other chemistries, follow the guidelines from the chemistry provider.

1. Breslauer *et al.,* 1986*. Proc. Nat. Acad. Sci.* 83, 3746-50.
2. Chester & Marshak, 1993. *Analytical Biochemistry* 209, 284-290.