

NEWS on diagnostics

2019 PCR Special Edition (3)



Welcome to the next volume of News on Diagnostics. This series of special editions covers the various steps in PCR. Volume 3 covers RNA/DNA amplification.

Volumes 1 and 2 cover sample collection and nucleic acid extraction & purification, respectively.

Download past issues at SigmaAldrich.com/newsdiagnostics

Amplification

DNA and RNA amplification via PCR is a fast, accurate, and sensitive way to produce large amounts of specific genomic sequences for further analysis. Despite its wide spread usage in research and applied laboratory settings, the technique still requires finesse and vigilance as variation in reagent quality, primer design, and operating parameters can significantly reduce copy yield and fidelity.

We offer a wide variety of reliable and precise Polymerase Chain Reaction (PCR) reagents, kits and tools to meet your experimental needs at any stage of development or manufacturing. Our products vary from routine to enhanced, so that you find the level of fidelity, speed, and accuracy you need for every PCR assay.

DNA polymerases

There is a wide selection of polymerases used today and our range of polymerases is customised to meet your End-Point PCR, qPCR, or RT-PCR needs. Use our online selection guide to narrow your choices and suggest the best product for your individual application.

For other search filter combinations, please visit our PCR selection guide at

SigmaAldrich.com/pcrselectionguide

Whether you're new to PCR or looking for a refresher, view our range of educational web-based seminars on qPCR at SigmaAldrich.com/qpcr-miqe-seminars

Product #	Product Name	HotStart	Color	Contents	Brand
D4812	REDAccuTaq ² LA DNA Polymerase	No	Red	• Enzyme • Optimized 10x Buffer	Sigma-Aldrich
D6045	AccuTaq ² LA DNA Polymerase	No	Clear	• Enzyme • Optimized 10x Buffer • DMSO	Sigma-Aldrich

PCR Master Mix Calculator

Performing calculations for large scale PCR reactions can be cumbersome and tedious. Ensure your success of scaled up reactions by using the PCR MasterMix Calculator at

SigmaAldrich.com/pcr-mastermix-calculator

This online tool will calculate the amounts of components needed to create your PCR Master Mix

PCR Master Mix Calculator

Composition of PCR reaction PCR MasterMix Formulation for [] PCR reactions

Template DNA	[] μ l	Template DNA	[] μ l
PCR Buffer	[] μ l	PCR Buffer	[] μ l
Forward Primer	[] μ l	Forward Primer	[] μ l
Reverse Primer	[] μ l	Reverse Primer	[] μ l
dNTP mix	[] μ l	dNTP mix	[] μ l
DNA Polymerase	[] μ l	DNA Polymerase	[] μ l
PCR grade Water	[] μ l	PCR grade Water	[] μ l
Total Number of Reactions	[]	Total PCR Reaction Volume	[] μ l
		TOTAL VOLUME	[] μ l

Reset Calculate Print

Troubleshooting your PCR procedure

Our online troubleshooting guide supplies helpful hints and tips on the five most common problems associated with PCR.

- No product
- Misincorporation or low fidelity
- Non-specific bands
- Smear bands
- Low yield

Read more on each of these symptoms at SigmaAldrich.com/working-with-pcr



Download our Technical Guide to PCR Technologies at SigmaAldrich.com/PCR-Tec-Guide

Did You Know?

In challenging situations, e.g., where the objective is to detect very low copy numbers or small differences in target concentration, it is advisable to select and test several primer combinations and then combine with a suitable probe.

MystiCq[®] microRNA reagents

MicroRNA-Based RT-qPCR

MystiCq[®]

MystiCq[®] microRNA reagents provide a complete SYBR Green RT-qPCR workflow for quantifying expression in three steps:

1. Isolation
2. cDNA synthesis
3. Quantitation by amplification

Enjoy a wide, linear dynamic range of input RNA, predesigned assay primers, and easy to use ReadyMixes compatible with most qPCR instruments. Choose from over 1400 pre-designed and wet lab primer pairs designed to target only mature microRNAs. Convert all microRNAs, allowing nearly unlimited readouts from a single sample. Select from three different products for isolation of microRNAs.

Check out our MystiCq[®] products and workflow at SigmaAldrich.com/MystiCq

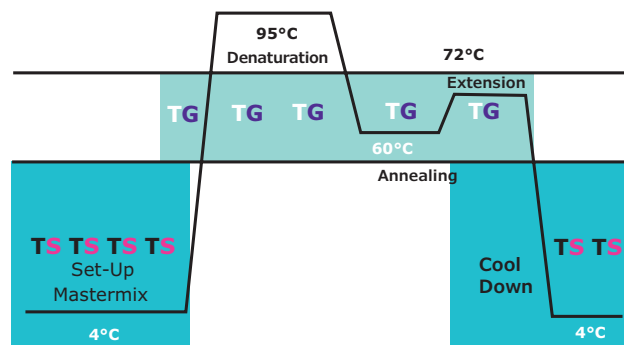
Thermagenix products

Whether you are working with DNA or RNA, increase the specificity and sensitivity of your PCR application by suppressing errors, before, during and after amplification by using these innovation Thermagenix products. These PCR additives make multiplexed diagnostic reactions more accurate and informative, and enhance the quality of all downstream applications, including next generation sequencing (NGS), deep sequencing, and Gibson assembly.

ThermaStop™	ThermaGo™	ThermaStop™-RT
For PCR that requires reduced non-specific amplification	Improves PCR specificity	Interacts with the reverse transcriptase at low temperatures to reduce priming errors
For highly multiplex PCR	Increases detection of low-copy number targets by suppressing amplification of non-specific products	Improves detection sensitivity and increases specific cDNA yield
For prevention of primer dimer formation	Reduces signal scatter in standard and digital PCR	Enhances accuracy of highly multiplexed RT-PCR assays
Eliminates undesired products after cooldown	Improves endpoint genotyping	Permits quantitatively accurate measurements of very low levels of RNA

ThermaStop™ (TS) is a reversible hot-start reagent that is compatible with thermostable 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

TS Acts Before and After, TG Acts During Amplification



is inhibited again with ThermaStop™ by cooling the reaction. Adding ThermaStop™ to the PCR reactions improves sensitivity and total product yield.

ThermaStop™ and ThermaGo™ (TG) act together during PCR amplification at different temperatures to increase specificity and prevent errors.

ThermaGo™ interacts with DNA polymerase during PCR in the annealing and extension steps to increase specificity and inhibit non-specific amplification.

ThermaStop™-RT is a proprietary additive for One-Step, or Two-Step Reverse Transcriptase-PCR assays containing gene-specific primers.

*Please note ThermaStop™-RT should not be used in combination with ThermaStop™ or ThermaGo™.

For more information, please visit SigmaAldrich.com/thermagenix



High performance Kapa Biosystems reagents for PCR and qPCR

Researchers can now turn to Merck as the exclusive, single-source supplier of the well-respected, high performance reagents developed for PCR and real-time PCR from Kapa Biosystems. This portfolio of reagents uses a high-throughput, directed evolution process that simulates natural selection in the lab, which allows the ability to engineer improvements to the structure and the function of enzymes.

Features and Benefits of Kapa PCR reagents:

- Used to evolve enzymes that are resistant to inhibitors, enabling direct PCR from samples such as blood and tissue

- Tolerant of the SYBR® Green dye, which improves sensitivity and reaction efficiency for qPCR assays
- High-throughput genotyping with shorter cycling times and higher yields and sensitivity

These products, paired with our precision biology tools, reagents, and technical expertise provides researchers with global access to key materials and process optimization across their workflow.

Visit SigmaAldrich.com/kapa for more information, including application notes, and full product portfolio we can supply.

