PerfeCTa® Multiplex qPCR SuperMix, Low ROX™

Cat No.	95108-050	Size:	50 x 50-µL reactions
	95108-200		200 x 50-µL reactions
	95108-01K		1000 x 50-µL reactions

Store at -25ºC to - 15°C protected from light

Description

PerfeCTa Multiplex qPCR SuperMix, Low ROX is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers, probe(s), and template for real-time quantitative PCR on Applied Biosystems 7500, 7500 Fast, ViiA7 or Stratagene MX series of real-time PCR systems. The system transcends multiplex limitations of conventional PCR master mixes, enabling unbiased amplification of up to five target sequences in a single tube. Suppression of low copy amplicons by high copy reference targets in the amplification is a common problem in multiplex PCR. This can skew, or mask the apparent representation and quantification of low copy target sequences. PerfeCTa Multiplex qPCR SuperMix, Low ROX delivers dynamic range and sensitivity to multiplexed qPCR that is comparable to that for singleplex qPCR probe assays without the need for limiting or variable primer concentrations.

The robust qPCR performance of Perfecta Multiplex qPCR SuperMix, Low ROX also overcomes suppression by common PCR inhibitors in blood or environmental samples, and is a highly sensitive reagent for quantification of low copy target sequences in challenging samples.

A key component of this supermix is AccuStart™ Taq DNA polymerase, which contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (2 minutes at 95ºC), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent to your specific instrument. PerfeCTa Multiplex qPCR SuperMix, Low ROX provides seamless integration on the Applied Biosystems 7500, 7500 Fast, ViiA™7 or Stratagene MX series of real-time PCR systems. These instruments utilize variable excitation wavelengths that are tuned to the each respective dye detection channel and provide superior sensitivities and dynamic ranges for multiplex probe applications. Your choice of probe reporter dyes and any optional internal reference dye must be matched to the excitation and emission optics of your particular instrument. Please consult the user manual for your real-time PCR system.

Components

PerfeCTa Multiplex qPCR Supermix, Low ROX	2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), AccuStart Taq DNA Polymerase, ROX Reference Dye (for 580-585 nm excitation), and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for Multiplex qPCR:

- The design of highly specific primers and probes is a critical and challenging aspect of successful multiplex qPCR. Each primer and probe should have similar thermodynamic properties to support efficient PCR amplification using a common temperature cycling program for all amplicons. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, primer pairs, and primer/probe combinations
- Amplicon size should be consistent for each target sequence and limited to approximately 65 - 100 bp.
- Limiting primer concentration for high copy genes is acceptable, but not required. A final concentration of 300 nM each primer and 100 to 250 nM probe is effective for most applications. Each probe for a multiplex assay should be labeled using dyes with minimal spectral overlap and non-fluorescent quencher compounds. Matching dyes with discrete fluorescent excitation and emission optima improves the accuracy of the multicomponenting, or dye deconvolution algorithms employed by the real-time PCR analysis software.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA) and dispense equal aliquots into each reaction tube. Add the DNA template to each reaction as the final step. Addition of samples as 5 to 10-µL volumes will improve assay precision.

Guidelines for qPCR continued:

- Suggested input quantities of template are: cDNA corresponding to 10 pg to 1 µg of total RNA; 100 pg to 1 µg genomic DNA
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 50-µL rxn.	Final Concentration
PerfeCTa Multiplex qPCR SuperMix	25 µL	1x
Forward primers	variable	100 – 500 nM each
Reverse primers	variable	100 – 500 nM each
Probes	variable	100 – 250 nM each
Nuclease-free water	variable	
Template(s)	5 – 10 µL	variable
Final Volume (µL)	50 µL	

Note: Reaction volume can be scaled from 10 to 50 µL depending on the reaction plate (i.e. 384-well vs. 96-well) and qPCR system. Scale all components proportionally to the desired final reaction volume. When performing qPCR in 384-well plates, use reaction volumes between 10 and 15 uL. High (20-uL) volumes can impair mixing of sample template with the reaction cocktail and can compromise qPCR performance and reproducibility

Cycling Protocol

Incubate complete reaction mix in a real-time thermal detection system as follows:

	Standard Protocol	**Fast Protocol**
Initial denaturation/activation	95ºC, 2 to 3 min	95ºC, 30s
PCR Cycling (35 to 45 cycles):	95ºC, 10 to 15 s	95ºC, 5s
	55 – 65ºC, 60s (collect and analyze data)	60ºC, 30s (collect and analyze data)

Full activation of AccuStart Taq DNA polymerase occurs within 30 seconds at 95ºC; however, optimal initial denaturation time is template dependent and will affect qPCR efficiency and sensitivity. Amplification of genomic DNA targets benefit from a prolonged initial denaturation step (5-10 min) to fully denature and fragment the template. This minimizes the potential for renaturation of long fragments and/or repetitive sequence regions that can impair replication of the target sequence by the PCR process.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa Multiplex qPCR SuperMix, Low ROX is functionally tested in a four-plex TaqMan qPCR using variable concentrations of one target sequence from 100 to 1 x 10^7 copies and 1 x 10^8 copies each of three other target sequences. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range (r^2 > 0.995) and a PCR efficiency > 90%.

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