PerfeCTa® MultiPlex qPCR SuperMix

Cat. No.	95063-050	Size:	50 x 50-µL reactions	Store at -20ºC protected from light
	95063-200		200 x 50-µL reactions	
	95063-01K		1000 x 50-µL reactions	

Description

PerfeCTa MultiPlex qPCR SuperMix is a 2X concentrated, ready-to-use reaction cocktail for real-time quantitative PCR (qPCR) that contains all components, except primers, probes and templates. 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 delivers dynamic range and sensitivity to multiplexed qPCR that is comparable to that for single-plex qPCR probe assays without the need for limiting or variable primer concentrations.

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 does not contain an internal reference dye to allow greater flexibility in your choice of reporter fluorophores and instrument platforms. Concentrated solutions of ROX™ Reference Dye or Low ROX Reference Dye are provided separately. In general, instruments that utilize variable excitation wavelengths that are tuned to the each respective dye detection channel 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	2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), AccuStart Taq DNA Polymerase, and stabilizers.
ROX Reference Dye (50X)	50X concentrated ROX solution optimized for Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, or StepOnePlus™
Low ROX Reference Dye (50X)	50X concentrated ROX solution optimized for systems using 580 nm to 585 nm excitation wavelength for the ROX dye channel: Applied Biosystems 7500, 7500 Fast, ViA™ 7, or Stratagene MX4000™, MX3005P™, MX3000P™

Storage and Stability

PerfeCTa Multiplex qPCR SuperMix is stable for 1 year when stored in a constant temperature freezer at -20ºC, protected from light. For convenience, it may be stored unfrozen at +2 to +8ºC for up to 6 months. Repeated freezing and thawing of the supermix is not recommended.

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.

Guidelines for qPCR continued:

- 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.
- 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 (2X)	25 µL	1x
Forward primers	variable	100 – 500 nM
Reverse primers	variable	100 – 500 nM
Probes	variable	100 – 250 nM
ROX or Low ROX Reference Dye (50X)	1 µL	*optional*
Nuclease-free water	variable	
Template(s)	5 – 10 µL	variable
Final Volume (µL)	50 µL	

Reaction Protocol

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

Initial denaturation:	95ºC, 2 to 3 min
PCR cycling (30-45 cycles:)	95ºC, 10 to 15 s
	55 – 65ºC, 60s (collect and analyze data)

Full activation of AccuStart Taq DNA polymerase occurs within 30 seconds at 95ºC. Initial denaturation times greater than 3 minutes are usually not required. However, amplification of gDNA targets may 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 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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