PerfeCTa® MultiPlex qPCR ToughMix®

Cat No.	95147-250	Size:	250 x 25-µL reactions (1 x 1.25 mL)	**Store at -25ºC to - 15°C protected from light**
	95147-01K		1000 x 25-µL reactions (4 x 1.25 mL)	
	95147-05K		5000 x 25-µL reactions (1 x 25 mL)	

Description

PerfeCTa MultiPlex qPCR ToughMix is a 5X concentrated, ready-to-use reaction cocktail for real-time quantitative PCR (qPCR) that overcomes many known inhibitors of PCR often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues. It contains all components, except primers, probes and templates. The 5X concentrated ToughMix allows addition of higher amounts of template and improved detection sensitivity with low concentration samples. PerfeCta MultiPlex qPCR ToughMix has been optimized to deliver maximum PCR efficiency, sensitivity, and specificity in reduced reaction volumes with fast cycle or conventional PCR cycling protocols.

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 that can skew, or mask the apparent representation and quantification of low copy target sequences. PerfeCTa MultiPlex qPCR ToughMix results in multiplexed qPCR with dynamic range and sensitivity that are comparable to single-plex qPCR probe assays without the need for limiting or variable primer concentrations.

A key component of PerfeCTa MultiPlex qPCR ToughMix is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is highly resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to PCR amplification.

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 ToughMix does not contain an internal reference dye to allow greater flexibility in your choice of reporter fluorophores and instrument platforms. 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 visit our Product Finder selection tool at www.quantabio.com to find the correct product for your real-time PCR system.

Components

PerfeCTa Multiplex qPCR ToughMix	5X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt.
Repeated freezing and thawing does not impair product performance.
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 25-µL rxn.	Final Concentration
PerfeCTa Multiplex qPCR SuperMix (5X)	5 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)	0.5 µL	*optional*
Nuclease-free water	variable	
Template(s)	5 – 10 µL	variable
Final Volume (µL)	25 µL	

Note: For smaller, or larger, reaction volumes scale all components proportionally.

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, 30s – 90s (collect and analyze data)

Full activation of the hot-start 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. The use of longer extension times may benefit some multiplexed qPCR assays. Optimal extension time should be empirically determined. We suggest using 60°C for 1 min as a general starting point.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa Multiplex qPCR ToughMix is functionally tested in a four-plex 5'-nuclease 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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