

# PerfeCta® MultiPlex qPCR ToughMix®

## qPCR multiplexing for tough samples

PerfeCta MultiPlex qPCR ToughMix is a multiplexing technology capable of overcoming many known inhibitors of PCR often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues.



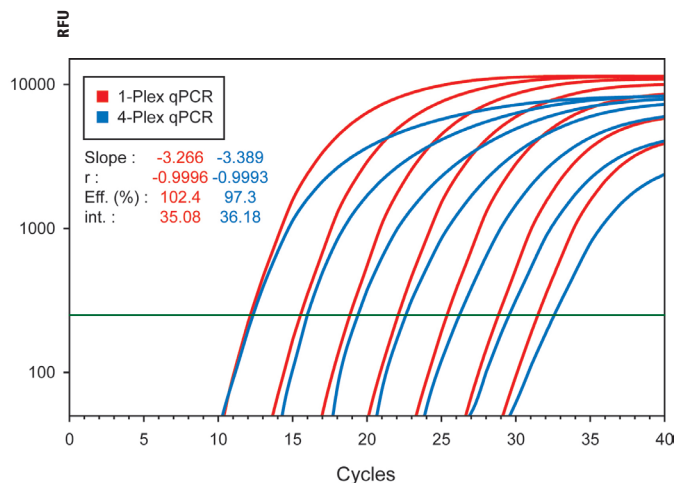
Quanta  
BIOSCIENCES™

### FEATURES AND BENEFITS

- **Tough Tested** - Overcomes common inhibitors including polysaccharides, heme/hemoglobin, humic acid, melanin
- **Flexible** - Use fast or standard qPCR cycling conditions
- **Broad dynamic range** - Reliable data from your precious samples every time

The PerfeCta MultiPlex qPCR ToughMix, capable of overcoming many known inhibitors, transcends the 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.

PerfeCta MultiPlex qPCR ToughMix is a 5X concentrated, ready-to-use reaction cocktail for real-time quantitative PCR (qPCR). 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.



#### High efficiency, high sensitivity multiplex qPCR results with PerfeCta MultiPlex qPCR ToughMix

Log-fold serial dilutions (10 to 1 E7 copies) of a plasmid containing the GAPDH gene, as well as no template controls, were amplified with PerfeCta MultiPlex qPCR ToughMix as either a single-plex qPCR, or a 4-target multiplexed qPCR that contained 1 E8 copies of 3 additional plasmid DNAs (ACTB, IL1beta, and TUBA). Quadruplicate reactions for each input quantity were carried out in 25- $\mu$ l volumes with 300 nM each primer and 150 nM each probe. Dual-labeled probes with non-fluorescent quenchers were from Biosearch Technologies. GAPDH was detected using a FAM-BHQ1 probe. ACTB; CAL Fluor Orange 560-BHQ1; IL1beta; CAL Fluor Red 610-BHQ2; TUBA; Quasar 670 - BHQ3. Single-plex qPCRs only contained the GAPDH primers and probe. Cycling was performed on a Bio-Rad CFX with the following protocol. 95C, 2 min; followed by 40 cycles of 95C, 10s; 58C, 90s. RFU data were exported to Excel, averaged for each replicate reaction series, and plotted.

## COMPONENTS

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.

PerfeCta Multiplex qPCR ToughMix	5X reaction buffer containing optimized concentrations of MgCl <sub>2</sub> , dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, and stabilizers.
PerfeCta Multiplex qPCR ToughMix ROX	5X reaction buffer containing optimized concentrations of MgCl <sub>2</sub> , dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, ROX reference dye and stabilizers.
PerfeCta Multiplex qPCR ToughMix Low ROX	5X reaction buffer containing optimized concentrations of MgCl <sub>2</sub> , dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, ROX reference dye and stabilizers.

## 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 important to match the appropriate reference dye to each specific optical detection system. 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. PerfeCta qPCR ToughMix, ROX contains an optimal concentration of a stabilized carboxy-X-rhodamine compound (ROX™) for instruments that use an excitation wavelength of ~490 nm and 605 to 610 nm emission channel for the reference signal. PerfeCta MultiPlex qPCR ToughMix Low ROX contains a ROX reference dye designed for qPCR systems using a 580 nm to 585 nm excitation wavelength for the ROX dye channel, such as Applied Biosystems 7500, 7500 Fast, ViA™ 7, or Stratagene MX4000™, MX3005P™, MX3000P™. It can also be used on real-time PCR systems that do not require a passive reference. Please visit our Product Finder selection tool at [www.quantabio.com](http://www.quantabio.com) to find the correct product for your real-time PCR system.

## STORAGE AND STABILITY

PerfeCta Multiplex qPCR ToughMix is stable for 2 years when stored in a constant temperature freezer at 20°C, protected from light. For convenience, it may be stored unfrozen at +2 to +8°C for up to 6 months. Repeated freezing and thawing of the ToughMix will not affect product performance.

## ORDERING INFORMATION

PRODUCT	Quanta Cat. No.	Pack Size (20uL Reactions)
<b>PerfeCta Multiplex qPCR ToughMix</b>	95147-250	250 x 25 ul rxns
	95147-01K	1000 x 25 ul rxns
	95147-05K	5000 x 25 ul rxns
<b>PerfeCta Multiplex qPCR ToughMix, ROX</b>	95148-250	250 x 25 ul rxns
	95148-01K	1000 x 25 ul rxns
	95148-05K	5000 x 25 ul rxns
<b>PerfeCta Multiplex qPCR ToughMix, Low ROX</b>	95149-250	250 x 25 ul rxns
	95149-01K	1000 x 25 ul rxns
	95149-05K	5000 x 25 ul rxns