PerfeCTa® qPCR ToughMix™, ROX™

Cat. No.	95113-250	Size:	250 x 20-uL reactions (2 x 1.25 mL)	Store at -20ºC
	95113-012		1250 x 20-µL reactions (10 x 1.25 mL)	protected from light
	95113-05K		5000 x 20-µL reactions (1 x 50 mL)	

Description

PerfeCTa qPCR ToughMix, ROX is a 2X concentrated ready-to-use reaction cocktail for PCR amplification of DNA templates that overcomes many known inhibitors of PCR often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues. It is a versatile and robust real-time qPCR reagent that provides maximum sensitivity and PCR efficiency with a variety of fluorogenic probe chemistries, including TaqMan® hydrolysis probes. PerfeCTa qPCR ToughMix, ROX contains all required reaction components, except primers, probe(s), and DNA template. The light blue color of the AccuVue™ tracer dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. It does not interfere with qPCR performance or affect the stability of the product.

A key component of PerfeCTa qPCR ToughMix, ROX 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 qPCR ToughMix, ROX is compatible with both fast and conventional PCR cycling protocols.

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 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. Please consult our Product Finder selection tool at www.quantabio.com to find the correct product for your real-time PCR system.

Components

PerfeCTa PCR ToughMix, ROX (2X):	2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, ROX reference dye, AccuVue blue qPCR dye, and stabilizers.

Storage and Stability

PerfeCTa qPCR ToughMix, ROX is stable for 2 years when stored in a constant temperature freezer at –20ºC. For convenience, it may be stored unfrozen at 2 to 8ºC for up to 6 months. After thawing, mix thoroughly before using.

Repeated freezing and thawing does not affect PCR performance.

Guidelines for qPCR:

- The design of highly specific primers and probes is a critical parameter for successful real-time PCR. 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, the primer pair, and primer/probe combinations. For best results, amplicon size should be limited to 65 - 200 bp. Optimal results may require titration of primer concentration between 100 and 900 nM. A final concentration of 300 - 400 nM each primer and 100 to 250 nM probe is effective for most applications. Increasing the concentration of the primer that initiates synthesis of the target strand that is complementary to the probe can improve fluorescent signal for some primer/probe systems.
- 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 2 to 5-µL volumes will improve assay precision.
- Suggested input quantities of template are: cDNA corresponding to 1 pg to 100 ng of total RNA; 10 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 20-µL rxn.	Final Concentration
PerfeCTa qPCR ToughMix, ROX (2X)	10 µL	1x
Forward primer	variable	100 – 900 nM
Reverse primer	variable	100 – 900 nM
Probe	variable	100 – 250 nM
Nuclease-free water	variable	
Template	2 – 5 µL	variable
Final Volume (µL)	20 µL	

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

PCR Cycling Protocol

	Fast 2-Step Cycling	Fast 3-Step Cycling	Standard Cycling
Initial denaturation:	95ºC, 30s *	95ºC, 30s *	95ºC, 2-3 min *
PCR cycling (30-45 cycles):	95ºC, 3 to 5s	95ºC, 3 to 5s	95ºC, 10 to 15s
		55 to 65ºC, 15s	
	60ºC, 20 to 30s †	68 to 72ºC, 10s †	60ºC, 30 to 60s †

The appropriate step for fluorescent data collection varies for different probe assay formats. Data collection for 5'-nuclease probe assays (TaqMan probe) should be carried out at the end of the extension step. Use the annealing step for data collection with hybridization probe assays (HybProbe® FRET hybridization probes, Molecular Beacons, Solaris® qPCR Assays, Scorpions® primers, etc.).
End-point analysis should be carried out at a suitable temperature for your detection probe chemistry.

* Full activation of the DNA polymerase occurs within 10 seconds at 95ºC; however, optimal initial denaturation time is *template dependent* and will affect qPCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled plasmid DNA targets may require 5 to 10 min at 95ºC to fully denature and fragment the template. Short double-stranded DNA template (PCR product) or single-stranded DNA template, such as cDNA, may require as little as 1s at 95ºC. Use 30s at 95ºC as a general starting point.

† Extension time is dependent upon amplicon length and the minimal data collection time requirement for your qPCR instrument. Use 30s at 60ºC as a general starting point. Some assay designs and/or detection chemistries may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set and real-time instrument.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa qPCR ToughMix, ROX is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($R^2 > 0.990$) with a 2-fold discrimination of starting template and a PCR efficiency > 95%.

Limited Label Licenses

The use of certain types of fluorogenic probes with 5' nuclease assays may be covered by U.S. Patent No. 5,538,848, owned by Life Technologies, Corporation. Purchase of this product does not convey rights to practice the methods claimed in U.S. Patent No. 5,538,848, and a license to practice those methods must be obtained from Life Technologies, 850 Lincoln Center Drive, Forest City, California 94404, or by purchase of fluorogenic probes from an authorized source.

Use of other hybridization probes may be covered by other patents and specific licenses may be required for each technology which is not conveyed by purchase of this product. Consult your probe provider for patent and license information related to each probe chemistry and their use.

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The use of hot start DNA polymerase in this kit is licensed to Quanta BioSciences, under U.S. Patent Nos. 5,338,671, 5,587,287, and foreign equivalents. Purchase of this kit provides the purchaser with a sublicense for research use only.

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Intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.