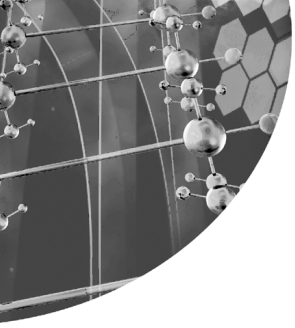

RealMasterMix™ Fast Probe ROX™ Kit

For quantitative fast-cycling real-time PCR using sequence-specific probes

Product specifications

Product description

RealMasterMix Fast Probe ROX Kit is a 2X concentrated, ready-to-use reaction mix that contains all required reaction components, except primers, probe(s) and template for real-time quantitative PCR systems using sequence-specific probes. The light blue color of the RealBlue dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting and mixing errors. The RealBlue dye does not interfere with qPCR performance.

Product limitations

RealMasterMix Fast Probe ROX Kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

Materials supplied

Kit	Order No	Size
RealMasterMix Fast Probe ROX Kit - 250 Rxn	2200760	2 x 1.25 ml
RealMasterMix Fast Probe ROX Kit - 2500 Rxn	2200770	20 x 1.25 ml

The RealMasterMix Fast Probe ROX Kit contains optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), SpeedAB *Taq* Polymerase, ROX reference dye, RealBlue dye, and stabilizers.

Storage and stability

The RealMasterMix Fast Probe ROX Kit is shipped on dry ice, but retains full activity at 20°C for up to 2 months.

RealMasterMix Fast Probe ROX Kit is stable until the expiration date indicated on the kit label if it is stored in a constant temperature freezer at -20°C and protected from light. The product can be stored at 2-8°C, but this will reduce the shelf life of the product to one fourth.

After thawing, mix thoroughly by gently vortexing before using. The product retains full functional performance after repeated free-thaw cycles.

Safety information

All due care and attention should be exercised in the handling of this product. We recommend all users of 5 PRIME products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. Specifically, always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals.

Neither of the vials contains hazardous substances in reportable quantities. The usual precautions taken when handling chemicals should be observed. Used reagents can be disposed of in waste water in accordance with local regulations. In case of eye contact, flush eyes with water. In case of skin contact, wash off with water. In case of ingestion, seek medical advice.

Additional safety information is available from www.5PRIME.com in material safety data sheets (MSDSs) for 5 PRIME products and 5 PRIME product components.

Quality assurance

5 PRIME products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- → **Component testing:** each component is tested to ensure the composition and quality meet stated specifications.
- → **Performance testing:** each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from www.5PRIME.com. Certificate of analysis sheets for 5 PRIME products and 5 PRIME product components can be obtained on request.

Product warranty

5 PRIME is committed to providing products that improve the speed, ease-of-use and quality of enabling technologies. 5 PRIME guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use.

This warranty is in place of any other warranty or guarantee, expressed or implied, instituted by law or otherwise. 5 PRIME provides no other warranties of any kind, expressed or implied, including warranties of merchantability and fitness for a particular purpose. Under no circumstance shall 5 PRIME be responsible for any direct, indirect, consequential or incidental damages or loss arising from the use, misuse, results of use or inability to use its products, even if the possibility of such loss, damage or expense was known by 5 PRIME.

5 PRIME distributors

A complete list of 5 PRIME distributors is available from www.5PRIME.com.

Protocol

Product principle

The RealMasterMix Fast Probe ROX Kit provides rapid real-time quantification of DNA and cDNA targets in an easy-to-handle format. RealMaster Mix Fast Probe ROX Kit can be used for fast, or conventional, cycling protocols.

Ultimate sensitivity and maximum PCR efficiency using a variety of fluorogenic probe chemistries, including TaqMan® hydrolysis probes, are achieved by the use of proprietary buffer, stabilizers, and SpeedAB *Taq* Polymerase. The SpeedAB *Taq* Polymerase contains a proprietary mixture of monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step (> 48 h at RT). For efficient extension kinetics and maximum yield the rapid recovery of fully active, unmodified *Taq* DNA Polymerase is critical. The SpeedAB *Taq* Polymerase is fully inactive at room temperature and is activated instantaneous at 95°C. This enables convenient reaction set up at room temperature with specific and efficient primer extension during the cycling. Replication of fragments up to 200 bp is complete in less than 20s at 60°C.

The SpeedAB *Taq* Polymerase is free of detectable *E. coli* DNA[§].

It is critical to match the appropriate reference dye to each specific optical detection system, because different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. The RealMasterMix Fast Probe ROX Kit 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.

[§] Testing of bulk enzyme for residual *E. coli* genomic DNA is validated to be less than 1 copy / unit.

Reagent	Rxns	Order No	Compatible Real-Time PCR Systems
RealMasterMix Fast Probe Kit	250 2500	2200740 2200750	Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™; Cepheid SmartCycler®; Eppendorf Mastercycler® ep realplex, realplex 2 s; Illumina Eco qPCR, Qiagen /Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; Roche Applied Science LightCycler™ 480; Thermo Scientific PikoReal Cycler
RealMasterMix Fast Probe ROX Kit	250 2500	2200760 2200770	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™
RealMasterMix Fast Probe Low ROX Kit	250 2500	2200780 2200790	Applied Biosystems 7500, 7500 Fast, ViiA™7; Stratagene MX4000™, MX3005P™, MX3000P™

Guidelines for Fast Cycle qPCR

- The single most important parameter for successful real-time PCR is the design of highly specific primers and probes. In order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combination the use of computer aided primer design programs is beneficial. For best results, amplicon size should be limited to 65 – 200 bp. A final concentration of 300 nM for each primer and 100 to 250 nM probe is effective for most reactions. However, optimal results may require titration of primer concentration between 100 and 900 nM.
- To reduce pipetting errors and maximize assay precision the preparation of a reaction cocktail is recommended. Dispense equal aliquots into each reaction tube. After assembling the reaction cocktail with all required components except sample template (genomic DNA or cDNA), add the DNA template to each reaction as the final step. Addition of samples as 2 to 5 µL volumes will improve assay precision.
- Recommended input quantities of template for cDNA corresponding to 1 pg to 100 ng of total RNA; or 10 pg to 1 µg genomic DNA.

Reaction setup

Component	Volume for 20 µl Rxn	Final Concentration
RealMasterMix Fast Probe ROX Kit (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	

Final reaction volume may vary from 10 to 50 µl, scale all components proportionally. After sealing each reaction, vortex gently to mix contents and centrifuge briefly to collect components at the bottom of the reaction tube.

PCR cycling conditions

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

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

* Although, the full activation of theSpeedAB *Taq* Polymerase occurs within 1 second at 95ºC the qPCR efficiency and sensitivity will be affected also by the optimal denaturation time, which is template dependent. 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, may require as little as 1 s at 95ºC. Use 30 s at 95ºC as a general starting point.

† Extension depends on amplicon length and minimal data collection time requirement for your qPCR instrument. Use 30 s at 60°C 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 or primer concentration may need to be empirically determined for any given primer set and real-time instrument.

Limited label licenses

The purchase of this product includes a limited, non-transferable right to use the purchased amount of the product to perform Applied Biosystems' patented Passive Reference Method for the purchaser' s own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser 's activities for a fee or other commercial consideration. is conveyed expressly, by implication, or by estoppel. This product is for research use only. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Licensed under U.S. Patent Nos. 5,338,671 and 5,587,287 and Corresponding Patents in Other Countries.