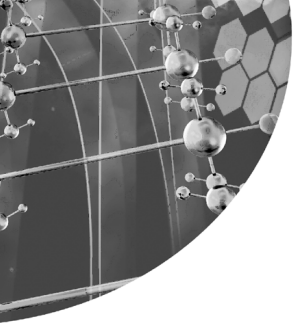

RealMasterMix™ Fast SYBR® Low ROX™ Kit

For quantitative fast-cycling real-time PCR using SYBR Green and Low ROX

Product specifications

Product description

RealMasterMix Fast SYBR Low ROX Kit is a 2X concentrated, ready-to-use reaction mix that contains all components, except primers and template for real-time quantitative PCR systems using SYBR Green on Applied Biosystems 7500, 7500 Fast, ViiA™ 7, or Stratagene MX series of real-time PCR systems.

Product limitations

RealMasterMix Fast SYBR Low 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 SYBR Low ROX Kit - 250 Rxn	2200860	2 x 1.25 ml
RealMasterMix Fast SYBR Low ROX Kit - 2500 Rxn	2200870	20 x 1.25 ml

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

Storage and stability

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

RealMasterMix Fast SYBR Low 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 4°C, but this will reduce the shelf life of the product by half.

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 SYBR Low ROX Kit provides rapid real-time quantification of DNA and cDNA targets in an easy-to-handle format. Maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional, cycling protocols with SYBR Green qPCR are achieved by the use of a unique combination of proprietary buffer, stabilizers, and SpeedAB *Taq* Polymerase. The fluorescent dye SYBR Green in the master mix enables the analysis of many different targets without having to synthesize target-specific labeled probes. The buffer also contains ROX dye, which allows fluorescence normalization on cycler Applied Biosystems 7500, 7500 Fast, ViiA™ 7, Stratagene MX4000™, MX3005P™ and MX3000P™.

SYBR Green I binds to and detects any dsDNA generated during amplification. Therefore highly specific amplification is crucial to successful qPCR with SYBR Green I dye. 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 20 s at 60°C.

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

It is critical to match the appropriate qPCR reagent to your specific instrument, because different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations.

[§] 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 SYBR Kit	250	2200820	Bio-Rad CFX96™, CFX384™, Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex; Roche Applied Science LightCycler® 480
	2500	2200830	
RealMasterMix Fast SYBR ROX Kit	250	2200840	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
	2500	2200850	
RealMasterMix Fast SYBR Low ROX Kit	250	2200860	Applied Biosystems 7500, 7500 Fast, ViiA™ 7, Stratagene MX4000™, MX3005P™, MX3000P™
	2500	2200870	
RealMasterMix Fast SYBR iQ Kit	250	2200880	Bio-Rad iCycler iQ®, iQ™5, MyiQ™
	2500	2200890	

Guidelines for Fast Cycle SYBR Green qPCR

- → The single most important parameter for successful real-time PCR with SYBR Green I dye is the design of highly specific primers. In order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer and the primer pair the use of computer aided primer design programs is beneficial. Although, RealMasterMix Fast SYBR Low ROX Kit can readily amplify fragments between 400 and 500 bp, amplicon size should be limited to less than 150 bp to take full advantage of fast cycling protocols. A final concentration of 300 nM for each primer is effective for most reactions. However, optimal results may require titration of primer concentration between 100 and 500 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 (genoic DNA or cDNA), add the DNA template to each reaction as the final step. Addition of samples as 5 to 10 µl volumes will improve assay precision.
- → Recommended input quantities of template for cDNA corresponding to 1 pg to 100 ng of total RNA or 100 pg to 100 ng genomic DNA.

Reaction setup

Component	Volume for 20 µl Rxn	Final Concentration
RealMasterMix Fast SYBR ROX Kit (2X)	10.0 µl	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
Nuclease-free water	variable	
Template	5 – 10 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	
Collect data at end of extension step	60ºC, 20 to 30 s[†]	68 to 72ºC, 10 s[†]	60ºC, 30 to 60 s[†]
Melt Curve (dissociation stage)	Refer to instrument instructions (optional)		

* Although, the full activation of SpeedAB *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. Some primer sets 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