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Not for use in diagnostic procedures.

Print

Roche

FastStart SYBR Green Master

Cat. No. 04 673 484 001 5 ml (4 × 1.25 ml; 500 × 20 µl reactions)
Cat. No. 04 673 492 001 50 ml (10 × 5 ml; 5,000 × 20 µl reactions)

Version 09
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Store at −15 to −25°C

⚠ Keep away from light

2× concentrated ready-to-use hot start master mix for qPCR and two-step qRT-PCR using the SYBR Green I detection format on real-time PCR instruments (except the LightCycler® Instruments)

1. What this Product Does

Contents

The FastStart SYBR Green Master is a ready-to-use, 2× concentrated master mix that contains all the reagents (except primers and template) needed for running quantitative, real-time DNA detection assays, including qPCR and two-step qRT-PCR, in the SYBR Green I detection format.

Storage and Stability

If stored at −15 to −25°C the master mix is stable until the expiration date printed on the label. For short-term storage (up to 3 months) the master may be stored at +2 to +8°C.

The reagent is shipped on dry ice.

⚠ Keep the master away from light.

⚠ Avoid repeated freezing and thawing.

The complete PCR mix *(i.e.,* FastStart SYBR Green Master supplemented with primers and template) is stable for up to 24 hours at +15 to +25°C.

⚠ Keep the PCR mix away from light.

Application

The FastStart SYBR Green Master is a ready-to-use reagent mix that simplifies the preparation of reactions for qPCR and two-step qRT-PCR, using the intercalating SYBR Green I Dye for DNA detection and analysis. In combination with a real-time PCR instrument and suitable PCR primers, FastStart SYBR Green Master allows sensitive detection and quantification of defined DNA sequences.

⚠ Do not use this product on the LightCycler® Instruments.

In principle, the FastStart SYBR Green Master can be used for the amplification and detection of any DNA or cDNA target, including those that are GC-rich or GC-poor. However, you would need to adapt your detection protocol to the reaction conditions of the particular real-time PCR instrument in use and design specific PCR primers for each target. See the instruction manual of your real-time PCR instrument for general recommendations.

⚠ The mix is designed for optimal amplification of targets up to 500 bp long. Do not use the mix to amplify longer targets.

FastStart SYBR Green Master offers convenience and ease-of-use because addition of $MgCl_2$ to the reaction mixture is not necessary, thus avoiding time-consuming optimization steps.

The mix contains dUTP, so that it may be used with Uracil-DNA Glycosylase to prevent false positives arising from carry-over contamination, *i.e.,* contamination with amplified DNA.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform quantitative real-time PCR assays with the FastStart SYBR Green Master include:

- standard laboratory equipment
  - nuclease-free, aerosol-resistant pipette tips
  - pipettes with disposable, positive-displacement tips
  - sterile reaction tubes for preparing master mixes and dilutions
  - standard benchtop microcentrifuge
  - Water, PCR Grade*
- for first-strand cDNA synthesis
  - Transcriptor First Strand cDNA Synthesis Kit*
- for real-time PCR
  - PCR reaction vessels (*e.g.,* optical tubes or microplates)
  - sequence-specific primers
- for carry-over prevention (optional)
  - LightCycler® Uracil-DNA Glycosylase*

2. How to Use this Product

2.1 Before You Begin

General

The optimal reaction conditions (concentration of template DNA and PCR primers, incubation temperatures and times, cycle number) depend on the specific template/primer system and must be determined individually.

Sample Material

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids use:
  - either MagNA Pure 96 Instrument, the MagNA Pure LC Instrument, or the MagNA Pure Compact Instrument together with a dedicated nucleic acid isolation kit (for automated isolation) or
  - a High Pure nucleic acid isolation kit (for manual isolation).

  For details see the Roche Applied Science Biochemicals catalog or home page, www.roche-applied-science.com.
- Use up to 250 ng complex genomic DNA or 50 ng cDNA.

Primers

Use PCR primers at a final concentration of 0.1 to 0.4 µM. The recommended starting concentration is 0.3 µM each.

⚠ Always use equimolar primer concentrations.

The design of the PCR primers determines amplicon length, melting temperature, amplification efficiency, and yield. Primer design may also depend on the choice of PCR program (two-step versus three-step protocol).

Several programs for primer design are freely available or provided by the suppliers of real-time PCR instruments (*e.g.,* PrimerExpress). Alternatively, such programs are available to the public on the web for free (*e.g.,* Primer3).

If you already plan to validate your results by a hydrolysis probe (5´ nuclease) assay later on, select primers with a T_m of +58 to +60°C. You can also evaluate your SYBR Green I assays using the pretested probes provided by the Universal ProbeLibrary available from Roche Applied Science. Appropriate assays, *i.e.,* PCR primers with their corresponding Universal ProbeLibrary probe, are designed by consulting the online ProbeFinder software availabe at www.universalprobelibrary.com.

Negative Control

To detect DNA contamination, always include a negative control in each run. To prepare this control, replace template DNA with Water, PCR Grade.

Reaction Volume

Various reaction volumes of the FastStart SYBR Green Master can be used. Please refer to the recommendations from the supplier of the real-time PCR instrument for suitable volumes and tubes/plates.

2.2 Procedure

Preparation of the PCR Mix

For each 50 µl reaction, prepare the following reaction mix:

❶ ▪ Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
▪ Mix carefully by pipetting up and down and store on ice.

❷ Prepare a 100× conc. solution of the PCR primers.

❸ In a 1.5 ml reaction tube on ice or the well of a PCR microplate, prepare the PCR Mix for one 50 µl reaction by adding the following components in the order mentioned below:

Component	Volume [a)]	Final conc.
FastStart SYBR Green Master	25 µl	1×
Forward primer (30 µM)	0.5 µl	300 nM
Reverse primer (30 µM)	0.5 µl	300 nM
Water, PCR Grade	19 µl	
Total Volume	**45 µl**	

a) To prepare the PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by z, where z = the number of reactions to be run + one additional reaction.

❹ ▪ Mix carefully by pipetting up and down. Do not vortex.
▪ Pipet 45 µl PCR mix into each PCR reaction vessel or well of a PCR microplate (depending on your real-time PCR instrument).

❺ ▪ Add 5 µl of the DNA (up to 250 ng) or cDNA.
In initial experiments to determine the optimum amount of cDNA template, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel. Too much DNA may inhibit the PCR.
▪ Mix carefully by pipetting up and down.

❻ According to the instructions supplied with your instrument, prepare the tubes or microplates for PCR (*e.g.*, seal tubes with optical tube caps or the plate with self-adhesive foil).

Performing PCR

⚠ For best results, be sure your instrument is correctly calibrated. Set the detection channel in the real-time PCR cycler to either SYBR Green or FAM (*i.e.,* 530 nm).

❶ A typical temperature profile is given for the Applied Biosystems PRISM 7700 SDS. Otherwise, follow the instruction manual of your instrument supplier:

Cycles	Analysis Mode	Target Temperature	Hold Time	Remarks
1 (optional)	None	50°C	2 min	Only if UNG had been added for carry-over prevention
1	None	95°C	10 min	Activation of FastStart Taq DNA Polymerase
40	Quantification	95°C primer dependent (typically 58 to 60°C) 72°C	15 sec 30 to 60 sec 30 to 60 sec	Amplification and real-time analysis

❷ Place your tubes or plate in the instrument and start the reaction.

❸ At the end of the reaction, follow instrument instructions for quantification/analysis.

2.3 Related Procedures

Prevention of Carry-Over Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over prevention technique involves incorporating deoxyuridine triphosphate into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template. Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

dUTP is a component of the FastStart SYBR Green Master.

⚠ Perform prevention of carry-over contamination with LightCycler® Uracil-DNA Glycosylase*. Add 1.25 U per 50 µl PCR reaction. Proceed as described in the Instructions for Use.

Two-step RT-PCR

FastStart SYBR Green Master can also be used to perform two-step RT-PCR. In two-step RT-PCR, the reverse transcription of RNA into cDNA is separated from the other reaction steps and is performed outside the real-time PCR instrument. Subsequent amplification and online monitoring is performed according to the standard real-time PCR procedure, using the cDNA as the starting sample material. Transcriptor First Strand cDNA Synthesis Kit* is recommended for reverse transcription of RNA into cDNA. Synthesis of cDNA is performed according to the detailed instructions provided with the kit.

3. Troubleshooting

Problem	Cause	Recommendation
No amplification detectable **and** no band in gel analysis	▪ error in PCR program (*e.g.,* activation step omitted)	▪ Control and adjust PCR program
	▪ pipetting errors (*e.g.*, DNA not added)	▪ Repeat the experiment and check carefully
	▪ amplicon length too high	▪ Design other primer
	▪ inhibitory effects of impurities	▪ Repeat isolation of your template
	▪ bad design of primers	▪ Design other primer
Fluorescence varies within a run	▪ instrument not correctly calibrated ▪ variations in pipetting	▪ Recalibrate instrument
High back ground in the negative (no template) control	Contamination	▪ Remake or replace critical solutions (*e.g.*, water) ▪ Clean lab bench ▪ Use UNG to prevent carry-over contamination

4. Additional Information on this Product

How this Product Works

The FastStart SYBR Green Master contains the FastStart Taq DNA Polymerase for hot-start PCR to improve specificity and sensitivity of the PCR by minimizing the formation of non-specific amplification products (1,2,3,4). This enzyme delivers excellent results thanks to its special enzyme design and optimized buffer system.
FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA Polymerase that shows no activity up to +75°C. The enzyme is active only at high temperatures, where primers no longer bind non-specifically. The enzyme is completely activated (by removal of blocking groups) in a single pre-incubation step (+95°C, 10 min) before cycling begins. Activation does not require the extra handling steps typical of other hot-start techniques.

Test Principle

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal (5). The SYBR Green I Dye intercalates into the DNA double helix (6). In solution, the unbound dye exhibits very little fluorescence; however, fluorescence (wavelength, 530 nm) is greatly enhanced upon DNA-binding. Therefore, during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated.

The basic steps of DNA detection by the SYBR Green I Dye during real-time PCR are:

① At the beginning of amplification, the reaction mixture contains the denatured DNA, the primers, and the dye. The unbound dye molecules weakly fluoresce, producing a minimal background fluorescence signal which is subtracted during computer analysis.

② After annealing of the primers, a few dye molecules can intercalate to the double strand. DNA binding results in a dramatic increase of the SYBR Green I molecules to emit light upon excitation.

③ During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

④ Fluorescence measurement at the end of the elongation step of every PCR cycle is performed to monitor the increasing amount of amplified DNA.

Quality Control

Each lot is tested for performance in qPCR using three templates: a GC-rich template, a GC-poor template and a long template (about 440 bp).

References

1 Chou, Q *et al.* (1992). Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nuc. Acid Res.* **20**, 1717-1723.

2 Kellogg, DE *et al.* (1994). TaqStart Antibody: hot-start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *BioTechniques* **16**, 1134-1137.

3 Birch, DE *et al.* (1996). Simplified hot start PCR. *Nature* **381**, 445-446.

4 PCR Manual 3rd edition, Roche Diagnostics.

5 Bustin, SA (*ed.*, 2004). A – Z of Quantitative PCR. IUL *Biotechnology Series*, **5.**

6 H. Zipper *et al.* (2004). Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. *Nuc. Acid Res.* **32**, e103.

5. Supplementary Information

5.1 Changes to Previous Version

- Disclaimer of License updated

5.2 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Numbered Instructions labeled ①, ②, *etc.*	Steps in a process that usually occur in the order listed
Numbered Instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.3 Abbreviations

In this document, the following abbreviations are used:

Abbreviation	Meaning
qPCR	quantitative real-time PCR
UNG	Uracil-DNA Glycosylase

5.4 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, http://www.roche-applied-science.com , and our Special Interest Sites including:

- The Universal ProbeLibrary: http://www.universalprobelibrary.com
- The MagNA Pure System family for automated nucleic acid isolation: http://www.magnapure.com
- Amplification – Innovative Tools for PCR: http://www.roche-applied-science.com/pcr
- DNA & RNA preparation – Versatile Tools for Nucleic Acid Purification: http://www.roche-applied-science.com/napure

Product	Pack Size	Cat. No.
FastStart TaqMan® Probe Master	2.5 ml (2 × 1.25 ml) 12.5 ml (10 × 1.25 ml) 50 ml (10 × 5 ml)	04 673 409 001 04 673 417 001 04 673 433 001
FastStart TaqMan® Probe Master (Rox)	2.5 ml (2 × 1.25 ml) 12.5 ml (10 × 1.25 ml) 50 ml (10 × 5 ml)	04 673 450 001 04 673 468 001 04 673 476 001
Transcriptor First Strand cDNA Synthesis Kit	50 reactions 100 reactions 200 reactions	04 379 012 001 04 896 866 001 04 897 030 001
LightCycler® Uracil-DNA Glycosylase	50 µl (100 U)	03 539 806 001
Water, PCR Grade	25 ml (25 × 1 ml) 25 ml (1 × 25 ml) 100 ml (4 × 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
High Pure PCR Template Preparation Kit	100 purifications	11 796 828 001
mRNA Isolation Kit	1 kit	11 741 985 001

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