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FastStart SYBR Green Master

Version: 10
Content version: November 2017

2x 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)

Cat. No. 04 673 484 001	5 ml 200 reactions of 50 µl final volume each 4 x 1.25 ml
Cat. No. 04 673 492 001	50 ml 2,000 reactions of 50 µl final volume each 10 x 5 ml

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	FastStart SYBR Green Master	Ready-to-use 2x master mix.	04 673 484 001	4 vials, 1.25 ml each
			04 673 492 001	10 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	FastStart SYBR Green Master	Store at −15 to −25°C. For short-term storage (up to 3 months), store at +2 to +8°C. ⚠ ***Keep protected from light.*** ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***The 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 protected from light.***

1.3. Additional Equipment and Reagents Required

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

For cDNA Synthesis

- Transcriptor First Strand cDNA Synthesis Kit*

For Real-Time PCR

- PCR reaction vessels (e.g., transparent PCR tubes or PCR microplates)
- Sequence-specific primers
- Water, PCR Grade*

For Prevention of Carryover Contamination (optional)

- LightCycler® Uracil-DNA Glycosylase*

1.4. Application

The FastStart SYBR Green Master is a ready-to-use 2x concentrated master mix that contains all 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. In combination with a real-time PCR instrument and suitable PCR primers, FastStart SYBR Green Master allows very sensitive detection and quantification of defined DNA sequences.

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

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template DNA (e.g., genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of PCR inhibitors.
- Use up to 250 ng complex genomic DNA or 50 ng cDNA.

For reproducible isolation of nucleic acids, we recommend:
- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

Control Reactions

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*.

Primers

Suitable concentrations of PCR primers range from 0.1 to 0.4 µM (final concentration in reaction). The recommended starting concentration is 0.3 µM each.

⚠ ***Always use equimolar primer concentrations.***

i *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, several programs are available free to the public on the web (e.g., Primer3).

i *If you plan to validate your results using a hydrolysis probe (5′ nuclease) assay, select primers with a Tm of +58 to +60°C. You can also evaluate your SYBR Green I assays using the pretested probes provided by the Roche Universal ProbeLibrary. Appropriate assays (i.e., PCR primers with their corresponding Universal ProbeLibrary probe), are designed by consulting the online ProbeFinder software.*

General Considerations

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.

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.

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.

2.2. Protocols

Preparation of the PCR Mix

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

1. 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.

2. Prepare a 100x conc. solution of the PCR primers.

3. 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:

Reagent	Volume [µl]	Final conc. [nM]
FastStart SYBR Green Master	25.0	1x
Forward primer (30 µM)	0.5	300
Reverse primer (30 µM)	0.5	300
Water, PCR Grade	19.0	–
Total Volume	45.0	

   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 plus one additional reaction.

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

5. 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.

6. Following the instructions supplied with your instrument, prepare the tubes or microplates for PCR (e.g., seal tubes with transparent 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).***

1 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 [°C]	Hold Time [hh:mm:ss]	Remarks
1 (optional)	None	50	00:02:00	Only if UNG was added for carryover prevention.
1	None	95	00:10:00	Activation of FastStart Taq DNA Polymerase.
40	Quantification	95 Primer dependent (typically 58 to 60°C) 72	00:00:15 00:00:30 – 00:00:60 00:00:30 – 00:00:60	Amplification and real-time analysis.

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

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

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This carryover 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 carryover contamination with LightCycler® Uracil-DNA Glycosylase. Add 1.25 U per 50 µl PCR reaction. Proceed as described in the Instructions for Use.***

3. Troubleshooting

Observation	Possible cause	Recommendation
No amplification detected and no band in gel analysis.	Error in PCR program (e.g., activation step omitted).	Adjust PCR program.
	Pipetting errors (e.g., DNA not added).	Repeat the experiment; check pipetting steps carefully.
	Amplicon length too long.	Redesign primer.
	Inhibitory effects of impurities.	Repeat isolation of your template.
	Poor primer design.	Redesign primer.
Fluorescence varies within a run.	Instrument not calibrated correctly.	Recalibrate instrument.
High background in the negative (no template) control.	Contamination	Remake or replace critical solutions (e.g., water).
		Clean lab bench.
		Use UNG to prevent carryover contamination.

4. Additional Information on this Product

4.1. Test Principle

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, therefore it may be used with Uracil-DNA Glycosylase to prevent false positives arising from carryover contamination (i.e., contamination with amplified DNA).

FastStart Taq DNA Polymerase

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 nonspecific amplification products (Chou, Q., et al., 1992; Kellogg, D.E., et al., 1994; Birch, D.E., et al., 1996). 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 nonspecifically. The enzyme is completely activated by removal of blocking groups in a single pre-incubation step (+95°C, 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

Detection of PCR Products

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal (Bustin, S.A., 2004). The SYBR Green I Dye intercalates into the DNA double helix (Zipper, H., et al., 2004). 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.

4.2. References

- Birch DE, Kolmodin L, Wong J, Zangenberg GA, Zoccoli MA, McKinney N, Young KKY - Simplified hot start PCR (1996) *Nature* **381** (6581), 445-446
- Bustin S A - A-Z of Quantitative PCR (2004) **5**, -
- Chou Q, Russell M, Birch DE, Raymond J, Bloch W - Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications (1992) *Nucleic Acids Research* **7**, 1717-1723
- Kellogg DE, Rybalkin I, Chen S, Mukhamedova N, Vlasik T, Siebert PD, Chenchika A - TaqStart antibody : hot start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase (1994) *BioTechniques* **16** (6), 1134-1137
- Zipper H, Brunner H, Bernhagen J, Vitzthum F - Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications (2004) *Nucleic Acids Research* **12**, -

4.3. 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).

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents , kits		
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001

5.4. Trademarks

FASTSTART, HIGH PURE, LIGHTCYCLER and MAGNA PURE are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All third party product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country. Country-specific contact information will be displayed.

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