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FastStart Universal SYBR Green Master (Rox)

 **Version: 07**

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

Cat. No. 04 913 850 001	4 x 1.25 ml 200 reactions of 50 µl final volume each
Cat. No. 04 913 914 001	10 x 5 ml 2,000 reactions of 50 µl final volume each

Store at –15 to –25°C

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	colorless	FastStart Universal SYBR Green Master (ROX)	Ready-to-use 2x master mix	04913850001 04913914001	4 vials, 1.25 ml each 10 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at -15 to -25°C , this product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	colorless	FastStart Universal SYBR Green Master (Rox)	<p>For short-term storage (up to 1 month), store product at $+2$ to $+8^{\circ}\text{C}$.</p> <p>⚠ Keep the FastStart Universal SYBR Green Master (ROX) away from light.</p> <p>⚠ Avoid repeated freezing and thawing.</p> <p>⚠ The complete PCR mix (i.e., FastStart Universal SYBR Green Master supplemented with primers, probe, and template) is stable for up to 24 hours at $+15$ to $+25^{\circ}\text{C}$. Keep the PCR mix away from light!</p>

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 PCR 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. General Information

1.4. Application

The FastStart Universal SYBR Green Master (ROX) is a ready-to-use, 2x concentrated master mix that contains all the reagents (except primers and template) needed for running real-time DNA detection assays, including qPCR and 2-step qRT-PCR, in the SYBR Green I detection format. It contains a special ROX reference dye making it suitable for all real-time PCR instruments on which ROX reference dye is needed for quantitative analysis.

In combination with a real-time PCR instrument, suitable PCR primers, and hydrolysis probe, FastStart Universal SYBR Green Master (ROX) 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 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 nucleic acid isolation kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

⚠ Store the template DNA either in water, PCR Grade or 5 – 10 mM Tris-HCl (pH 7.5 – 8.0). Avoid dissolving the template in TE buffer because EDTA chelates Mg²⁺.

Control Reactions

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

Primers

Use PCR primers at a final concentration of 0.1 – 0.4 μM. 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 (2-step versus 3- 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.

i *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 validate your SYBR Green I assays using the probes provided by the Universal ProbeLibrary available from Roche Life Science. Appropriate assays, *i.e.*, PCR primers with their corresponding Universal ProbeLibrary probe, are designed by consulting the online ProbeFinder software available at <http://www.universalprobelibrary.com/>.*

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 Universal SYBR Green Master (ROX) can be used. Please refer to recommendations from the supplier of the instrument for suitable volumes and tubes/plates.

ROX Reference Dye

In principle, real-time PCR instruments (except the LightCycler® Instruments) offer two different modes:

- Detection of SYBR Green I fluorescence in relationship to a reference dye (usually ROX)
- Detection of SYBR Green I fluorescence alone

The choice of mode depends on the instrument (*e.g.*, whether a channel for detecting the reference dye is available) and on the light source of the instrument (halogen versus laser).

The FastStart Universal SYBR Green Master (ROX) is supplemented with ROX reference dye and is proven to run on Applied Biosystems (ABI) instruments (the ABI PRISM 7000 Sequence Detection System, the ABI 7300 Real-Time PCR

2. How to Use this Product

System, the ABI 7500 Real-Time PCR Systems, the ABI 7700 Sequence Detection System, and the ABI PRISM 7900 HT Fast Real-Time PCR System) as well as on the Stratagene Mx3000P QPCR System without the need to adjust the ROX concentration.

- i** If you do not want to use the reference channel of your real-time PCR instrument or the instrument is not equipped with a reference channel, use the FastStart SYBR Green Master* (without ROX).
- i** If you use the Bio-Rad iCycler iQ5 Real-Time PCR Detection System use the FastStart SYBR Green Master* (without ROX) and apply the External Well Factor Plate procedure for determining the well factors. For details on how to perform the External Well Factor Plate procedure consult the Bio-Rad iCycler iQ5 Real-Time PCR Detection System Instruction Manual.

Two-Step RT-PCR

FastStart Universal SYBR Green Master (ROX) 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 PCR Master Mix

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

- 1** – Thaw the solutions and, for maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.
– Mix solutions carefully by pipetting them up and down, then store on ice.

⚠ If you are using the 5 ml or 50 ml vials of the FastStart Universal SYBR Green Master (ROX), mix the content by rotating the vials several times without shaking.

- 2** Prepare 100x conc. solutions (30 µM) of the PCR primers.

- 3** In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 50 µl reaction by adding the following components in the order listed below:

Reagent	Volume ⁽¹⁾ [50 µl reaction]	Final Conc.
FastStart Universal SYBR Green Master (ROX)	25 µl	1x
Forward Primer (30 µM)	0.5 µl	300 nM
Reverse Primer (30 µM)	0.5 µl	300 nM
Water, PCR Grade	19.0 µl	
Total Volume	45 µl	

- 4** – Mix the solution 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).

- 5** – Add 5 µl of template DNA (up to 250 ng total DNA or 50 ng cDNA).

- i** To determine the optimum amount of cDNA template in initial experiments, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel.
– Mix carefully by pipetting up and down.

- 6** 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).

⁽¹⁾ To prepare the PCR mix for more than one reaction, multiply the amounts in the "Volume" column by z, where z = the number of reactions to be run + one additional reaction.

Performing PCR

There are several different ways to program the PCR. Either two-step or three-step PCR programs will provide suitable experimental results. The amplicon should be short (approx. 150 bp) and the annealing/elongation temperature should be +60°C (e.g., a typical PCR protocol is 40 cycles of 95°C/15 s, followed by +60°C/1 min).

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

The table below shows an example standard PCR protocol.

i If you want to perform a fast qPCR protocol in 20 µl reaction on an ABI instrument equipped with a FastPlate (e.g., the ABI 7500 Fast Real-Time PCR System or ABI PRISM 7900 HT Fast Real-Time PCR System) apply the Hold Time given in brackets. This will reduce cycling time to about 1 hour.

1 Following the Operator's Manual of your instrument supplier, program the instrument with the following parameters:

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

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 and melting analysis.

⁽¹⁾ Hold Time when applying a fast PCR protocol

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This carryover prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix in this kit) 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.

i Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

i dUTP is a component of the FastStart Universal SYBR Green Master (ROX).

⚠ Perform prevention of carryover contamination with LightCycler[®] Uracil-DNA Glycosylase*. Add 1.25 – 2.5 U per 50 µl PCR reaction. Proceed as described in the Instructions for Use

3. Troubleshooting

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

4. Additional Information on this Product

4.1. Test Principle

The FastStart Universal SYBR Green Master (ROX) 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 a specific hydrolysis probe and PCR primers for each target. See the Operator's 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.

i FastStart Universal SYBR Green Master (ROX) offers convenience and ease-of-use because

- Adjustment of ROX concentration is **not** required in order to achieve the same performance on different real-time PCR instruments which need ROX for quantitative analysis.

- The addition of MgCl₂ to the reaction mixture is not necessary, thus avoiding time-consuming optimization steps.

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

FastStart Taq DNA Polymerase

The FastStart Universal SYBR Green Master (ROX) 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 (Chou, Q. et al., 1992; Kellogg, D.E. et al., 1994). 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.

Detection of PCR Products

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal (Bustin, S.A., ed., 2004). SYBR Green I 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 SYBR Green I 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. Additional Information on this Product

4.2. References

- Bustin S A (Ed. 2004) A-Z of Quantitative PCR, IUL Biotechnology Series, 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.
① ② ③ 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

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
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
High Pure PCR Template Preparation Kit	1 kit, up to 100 purifications	11 796 828 001
FastStart SYBR Green Master	5 ml, 4 x 1.25 ml, 200 reactions of 50 µl final volume each	04 673 484 001
	50 ml, 10 x 5 ml, 2,000 reactions of 50 µl final volume each	04 673 492 001
FastStart TaqMan® Probe Master	2 x 1.25 ml, 100 reactions of 50 µl final volume each	04 673 409 001
	10 x 1.25 ml, 500 reactions of 50 µl final volume each	04 673 417 001
	10 x 5 ml, 2,000 reactions of 50 µl final volume each	04 673 433 001
FastStart Universal Probe Master (Rox)	2 x 1.25 ml, 100 reactions of 50 µl final volume each	04 913 949 001
	10 x 1.25 ml, 500 reactions of 50 µl final volume each	04 913 957 001
	10 x 5 ml, 2,000 reactions of 50 µl final volume each	04 914 058 001

5. Supplementary Information

5.4. Trademarks

FASTSTART, HIGH PURE, LIGHTCYCLER, MAGNA PURE and TAQMAN 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.

