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FastStart TaqMan® Probe Master

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

Cat. No. 04 673 409 001	2 x 1.25 ml 100 reactions of 50 µl final volume each
Cat. No. 04 673 417 001	10 x 1.25 ml 500 reactions of 50 µl final volume each
Cat. No. 04 673 433 001	10 x 5 ml 2,000 reactions of 50 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	FastStart TaqMan® Probe Master	Ready-to-use 2x master mix.	04 673 409 001	2 vials, 1.25 ml each
			04 673 417 001	10 vials, 1.25 ml each
			04 673 433 001	10 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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 TaqMan® Probe 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 TaqMan® Probe Master supplemented with primers, probe, 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
- A hydrolysis probe (e.g., from the Universal ProbeLibrary sets)
- Water, PCR Grade*

For Prevention of Carryover Contamination (optional)

- LightCycler® Uracil-DNA Glycosylase*

1.4. Application

The FastStart TaqMan® Probe Master is a ready-to-use 2x concentrated master mix that contains all reagents (except primers, probe, and template) needed for running quantitative, real-time DNA detection assays, including qPCR and two-step qRT-PCR in the hydrolysis probe detection format. In combination with a real-time PCR instrument, suitable PCR primers, and a hydrolysis probe, FastStart TaqMan® Probe 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 primers range from 0.3 to 1.0 µM (final concentration).The recommended starting concentration is 0.9 µM each.

⚠ ***Always use equimolar primer concentrations.***
⚠ ***If you are using probes from the Universal ProbeLibrary, use 200 nM of each primer.***

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 free to the public on the web. For example, use the free online ProbeFinder software to design primers that may be paired with probes from the Universal ProbeLibrary.

Probe

The probe concentration should be significantly lower than the primer concentration. As a starting point, we recommend using 250 nM probe. However, suitable concentrations range from 100 nM to 300 nM.

⚠ ***To ensure a specific and sensitive assay, the probe must anneal to the DNA at a significantly higher temperature than the primers. Therefore, the Tm of the probe should be +68 to +70°C and the Tm of the primers approximtely +58 to +60°C.***
⚠ ***For maximum assay sensitivity, avoid placing a terminal G at the 5′ end of the probe because the fluorescent signal (arising after cleavage of the probe) is adversely affected by this terminal G.***

To ensure that the fluorescent reporter dye within the unreacted probe is quenched, the length of the probe should not exceed 28 nucleotides.
If you use probes from the Universal ProbeLibrary, start with a probe concentration of 100 nM. Set the annealing temperature to +60°C.

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 TaqMan® Probe Master can be used. Please refer to recommendations from the supplier of the instrument for suitable volumes and tubes/plates.

Real-Time PCR Instruments

⚠ ***Please read carefully.***

In principle, real-time PCR instruments (except the LightCycler® Instruments) offer three different modes:
- Detection of released signal in relationship to a reference dye (usually Rox).
- Detection of released signal in relationship to the quencher dye of the probe (e.g., TAMRA).
- Detection of released signal alone.

The choice of mode depends on the instrument (e.g., whether a channel for detecting the reference dye is available) and the light source of the instrument (halogen or laser).
The FastStart TaqMan® Probe Master is only available without Rox. If you use the Bio-Rad iCycler iQ5 Real-Time PCR Detection System, apply only the External Well Factor Plate procedure for determining the well factors. For determining the well factors, the Bio-Rad iCycler iQ5 Real-time PCR Detection System does not use Rox but fluorescein. Therefore, use the FastStart TaqMan® Probe Master in combination with the iCycler iQ5 Real-time PCR Detection System only. For details on how to perform the External Well Factor Plate procedure, consult the iCycler iQ5 Real-time PCR Detection System Instruction Manual.

i *Refer to the FastStart Universal Probe Master (Rox)* for instruments which use Rox as a reference dye.*

Two-Step RT-PCR

FastStart TaqMan® Probe 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 maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.
   – Mix solutions carefully by pipetting up and down and store on ice.

2. Prepare 100x conc. solutions of the PCR primers and the hydrolysis probe.

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 [µl]	Final conc. [nM]
FastStart TaqMan® Probe Master	25.0	1x
Hydrolysis Probe (25 µm)	0.5	250
Forward primer (90 µM)	0.5	900
Reverse primer (90 µM)	0.5	900
Water, PCR Grade	18.5	–
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 the solution 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 template DNA (up to 250 ng total DNA) 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.
   – 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

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

⚠ ***For best results, be sure the instrument is calibrated correctly.***

1. Following the instruction manual of the instrument supplier, program the instrument with the following parameters:

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	Dependent on the specific primer-probe combination.		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.

ℹ *dUTP is a component of the FastStart TaqMan® Probe 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.
No or low amplification detected but strong band in gel analysis.	PCR is working but the probe is poorly designed.	Redesign probe.
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 TaqMan® Probe 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 a specific hydrolysis probe and 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 TaqMan® Probe 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 carryover contamination (i.e., contamination with amplified DNA).

The FastStart TaqMan® Probe Master is fully compatible with probes from the Universal ProbeLibrary Sets.

FastStart Taq DNA Polymerase

The FastStart TaqMan® Probe 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

Real-time DNA detection assays based on the hydrolysis probe format (also known as 5′-nuclease assays) require a single, signal-generating probe that contains two labels, a fluorescent reporter dye at the 5′ end and a (fluorescent or dark) quencher label at or near the 3′ end (Holland, P.M., et al., 1991). When the probe is intact, the fluorescence signal is almost completely suppressed by the quenching label. When the probe is hybridized to its target sequence, it is cleaved by the 5′ → 3′ exonuclease activity of the FastStart Taq DNA Polymerase, which “unquenches” the fluorescent reporter dye. During each PCR cycle, more of the released fluorescent dye accumulates, boosting the fluorescence signal.

⚠ ***If you use the hydrolysis probe format for detection, you cannot perform a subsequent melting curve analysis. For melting curve analysis, we recommend using the FastStart SYBR Green Master*.***

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
- 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
- Holland PM, Abramson RD, Watson R, Gelfand DH - Detection of specific polymerase chain reaction product by utilizing 5′ → 3′ the exonuclease activity of Thermus aquaticus DNA polymerase (1991) *Proc Natl Acad Sci U S A* **16**, 7276-7280
- 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

4.3. Quality Control

Each lot is function 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
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

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.

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