For general laboratory use.

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LightCycler® FastStart DNA Master^{PLUS} HybProbe

Version: 12

Content version: December 2016

Easy-to-use hot start reaction mix (5x conc.) for PCR using the LightCycler® Carousel-Based System

Cat. No. 03 515 567 001	1 kit 480 reactions of 20 µl final volume each
Cat. No. 03 515 575 001	1 kit 96 reactions of 20 µl final volume each
Cat. No. 03 752 178 001	1 kit 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description		Catalog Number	Content
1a	white	LC FastStart DNA Master^{PLUS} HybProbe, Enzyme	Contains FastStart Taq DNA Polymerase, reaction buffer, $MgCl_2$, and dNTP mix (with dUTP instead of dTTP).	Ready-to-use hot start PCR Master after pipetting 10 µl from Vial 1a into one Vial 1b.	03 515 575 001	1 vial 1a and 3 vials 1b, for 3 vials, 128 µl each LC FastStart DNA Master^{PLUS} HybProbe, 5x conc.
					03 515 567 001	5 vials 1a and 15 vials 1b, for 15 vials, 128 µl each LC FastStart DNA Master^{PLUS} HybProbe, 5x conc.
1b	red	LC FastStart DNA Master^{PLUS} HybProbe, Reaction Mix		Ready-to-use hot start PCR Master after pipetting 50 µl from Vial 1a into one Vial 1b.	03 752 178 001	4 vials 1a and 12 vials 1b, for 12 vials, 640 µl each LC FastStart DNA Master^{PLUS} HybProbe, 5x conc.
2	colorless	LC FastStart DNA Master^{PLUS} HybProbe, Water, PCR Grade	To adjust the final reaction volume.		03 515 575 001	2 vials, 1 ml each
					03 515 567 001	7 vials, 1 ml each
					03 752 178 001	2 vials, 25 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.
Once the kit is opened, store the kit components as described in the following table:

Vial/Bottle	Cap	Label	Storage
1a	white	Enzyme	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
1b	red	Reaction Mix	
1 (after addition of 1a to 1b)	red	LC FastStart DNA Master^{PLUS} HybProbe, Master Mix, 5x conc.	▪ Store at −15 to −25°C. ▪ The prepared Master Mix can be aliquoted and stored at −15 to −25°C for a maximum of 3 months or at +2 to +8°C for a maximum of one week. ⚠ ***Avoid repeated freezing and thawing.***
2	colorless	Water, PCR Grade	Store at −15 to −25°C.

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

For PCR

- LightCycler® Carousel-Based System*
- LightCycler® Capillaries*

LightCycler® Capillaries (100 µl) can only be used with the LightCycler® 2.0 Instrument.

- Standard benchtop microcentrifuge containing a rotor for 2 ml reaction tubes

The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.

or

- LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousel (optional)
- LightCycler® Uracil-DNA Glycosylase* (optional)

For prevention of carryover contamination; see section **Prevention of Carryover Contamination***. Use LightCycler® Uracil-DNA Glycosylase in combination with LightCycler® FastStart DNA Masters only.*

- LightCycler® Color Compensation Set* (optional)

To perform color compensation when using the LightCycler® Red 640- and Cy5.5-labeled HybProbe probes in dual-color experiments in the same capillary, see section **Color Compensation***.*

1.4. Application

LightCycler® FastStart DNA Master^{PLUS} HybProbe is designed for PCR applications using the LightCycler® Carousel-Based System. The kit is ideally suited for hot start PCR applications.
The kit can also be used in conjunction with heat-labile Uracil-DNA Glycosylase to prevent carryover contamination during PCR.

1.5. Preparation Time

Assay Time

Procedure	Assay Time 20 µl reactions [min]	Assay Time 100 µl reactions [min]
PCR Setup	15	15
LightCycler® Carousel-Based System PCR run	45	90
Total Assay Time	**60**	**105**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template DNA suitable for PCR in terms of purity, concentration, and absence of PCR inhibitors.
- Use up to 50 ng complex genomic DNA or 10^1 to 10^{10} copies plasmid DNA per 20 µl reaction.

⚠ *If you are using a non-purified cDNA sample from reverse transcription, especially if it contains high background concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl (or less) of that sample in the reaction.*

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

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Water, PCR Grade (Vial 2).

Primers

Suitable concentrations of PCR primers range from 0.2 to 1 µM (final concentration). The recommended starting concentration is 0.5 µM each.

i *If amplification curves show the "Hook effect" (i.e., after an exponential rise, the fluorescence signal reaches a maximum, then significantly drops in later cycles), subsequent melting curve analysis often can be improved by asymmetric PCR. To perform asymmetric PCR, use a lower concentration of the primer that starts the amplicon strand competing with the probes for the complementary strand. Titrate this primer down to 1/10 of the other primers concentration in several steps and determine a ratio where the melting curves are better suited for analysis, while Cp values of the amplification curves are not shifted significantly.*

Probe

HybProbe Probes

Use the HybProbe probes at a final concentration of 0.2 µM each. In some cases, it might be advantageous to double the concentration of the LightCycler® Red-labeled probe to 0.4 µM.

i *For more information about HybProbe probes, see section Test Principle.*

Mg^{2+} Concentration

$MgCl_2$

Due to the optimized Reaction Mix (Vial 1b) of the LightCycler® FastStart DNA Master^{PLUS} HybProbe, the PCR is efficient and specific for almost all primer combinations without any $MgCl_2$ optimization.

General Considerations

Color Compensation

When using HybProbe probes that contain different red fluorophore labels in the same capillary, a previously generated color compensation file must be used to compensate for the crosstalk between the individual channels. A previously stored color compensation file can be activated during the LightCycler® Instrument run, or during data analysis after the run.

Although the optical filters of each detection channel of the LightCycler® Carousel-Based System are optimized for the different emission maxima of the fluorescent dyes, some residual crosstalk between the channels will occur unless corrected for with a color compensation file or object.

Color compensation is instrument-specific. Therefore, a color compensation file must be generated for each LightCycler® Carousel-Based Instrument.

No universal color compensation set is available for multicolor applications using a different dye combination than LightCycler® Red 640 and Cy5.5. Such assays have to use a customized color compensation object. You have to prepare a new color compensation object for each set of parameters.

For more information on the generation and use of a color compensation file, please see the LightCycler® Instrument Operator's Manual, the Special Interest Site for the LightCycler® Real-Time PCR Systems www.lightcycler.com, or the Instructions for Use of the LightCycler® Color Compensation Set .

Two-Step RT-PCR

LightCycler® FastStart DNA Master^{PLUS} HybProbe 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 PCR and is performed outside the LightCycler® Carousel-Based System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® Carousel-Based System procedure, using the cDNA as starting sample material. One of the following reagents is required for reverse transcription of RNA into cDNA:

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*

Synthesis of cDNA is performed according to the detailed instructions provided with the cDNA synthesis reagent.

Do not use more than 5 µl of undiluted cDNA template per 20 µl final reaction volume because greater amounts may inhibit PCR. For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount.

2.2. Protocols

LightCycler® Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® Carousel-Based System.

⚠ ***Program the LightCycler® Carousel-Based System before preparing the reaction mixes.***
A LightCycler® Carousel-Based System protocol that uses LightCycler® FastStart DNA Master^{PLUS} HybProbe contains the following programs:

- **Pre-Incubation** for activation of FastStart DNA polymerase and denaturation of the DNA
- **Amplification** of the target DNA
- **Melting Curve** for amplicon analysis: Optional, only needed for SNP or mutation detection
- **Cooling** the rotor and thermal chamber

ⓘ *For details on how to program the experimental protocol, see the LightCycler® 2.0 Instrument Operator's Manual.*

⚠ ***Set all other parameters not listed in the table below to 0.***
The following table shows the PCR parameters that must be programmed for a LightCycler® Carousel-Based System PCR run with the LightCycler® FastStart DNA Master^{PLUS} HybProbe.

LightCycler® Software Version 4.1		
Programs		
Setup	Setting	
Default Channel	**During the run:** Depending on the acceptor dye used for labeling the HybProbe probe, choose channel 610, 640, 670, or 705. **For analysis:** Divide by channel 530 for single-color experiments; divide by "Back 530" for dual- or multiple-color experiments (*e.g.,* 640/Back 530). For automated Tm Calling analysis, do not divide by channel 530 or "Back 530". ⓘ *Channel 610 and 670 are available on a LightCycler® 2.0 Instrument only.*	
Seek Temperature	30°C	
Max Seek Pos.	Enter the total number of sample positions the instrument should search.	
Instrument Type	"6 Ch." for LightCycler® 2.0 Instrument or "3 Ch." for LightCycler® 1.5 Instrument	
Capillar Size	Select "20 µl" as the capillary size for the experiment. (available only for LightCycler® 2.0 Instrument (6 channels))	
Programs		
Program Name	Cycles	Analysis Mode
Pre-Incubation	1	None
Amplification	45	Quantification
Melting Curve	1	Melting Curve
Cooling	1	None

LightCycler® Software Version 4.1				
Programs				
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate[1] [°C/s]	Acquisition Mode [per °C]
Pre-Incubation	95	00:10:00[2]	20	None
Amplification	95	00:00:15	20	None
	primer dependent[3]	00:00:05 – 00:00:20 (20 µl)[4] 00:00:30 – 00:00:45 (100 µl)	20	Single
	72[5]	00:00:05 - 00:00:30 [4,6]	20	None
Melting Curve	95	00:00:00	20	None
	40	00:00:30 (20 µl) 00:00:60 (100 µl)	5	None
	95	00:00:00	0.1	Continuous
Cooling	40	00:00:30	20	None

[1] Ramp Rate is 20°C/second, except where indicated.
[2] If high polymerase activity is required in early cycles, you can sometimes improve results by extending the pre-incubation to 15 minutes.
[3] For initial experiments, set the target temperature (*i.e.,* the primer annealing temperature) 5°C below the calculated primer Tm. Calculate the primer Tm according to the following formula, based on the nucleotide content of the primer: Tm = 2°C (A + T) + 4°C (G + C).
[4] For greater precision in target quantification experiments, it can be advantageous to choose longer annealing and extension times for the amplification cycles.
[5] If the primer annealing temperature is low (<55°C), reduce the Ramp Rate to 2-5°C/second.
[6] Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (*e.g.*, a 500 bp amplicon requires 20 seconds elongation time).

Preparation of the Master Mix

Prepare the 5x conc. Master Mix as described below.

1. Thaw one vial of Reaction Mix (Vial 1b).

2. Briefly centrifuge one vial Enzyme (Vial 1a) and the thawed vial of Reaction Mix.

3. – For Cat. Nos. 03 515 575 001 and 03 515 567 001, pipette 10 µl from Vial 1a into Vial 1b.
   – For Cat. No. 03 752 178 001, pipette 50 µl from Vial 1a into Vial 1b.

   Each Vial 1a contains enough enzyme for 3 Vials 1b.

4. Mix gently by pipetting up and down.

   Do not vortex.

5. Re-label Vial 1b with the new label (Vial 1: LC FastStart DNA Master^{PLUS} HybProbe, Master Mix, 5x conc.) provided with the kit.

   Always keep the Master Mix cool and protected from light.

Preparation of the PCR Mix

Prepare the PCR Mix as described below.

⚠ ***Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.***

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries into precooled centrifuge adapters or into a LightCycler® Sample Carousel in a precooled LC Carousel Centrifuge Bucket.

2. Prepare a 10x conc. solution that contains PCR primers and HybProbe probes.

3. Thaw the LC FastStart DNA Master^{PLUS} HybProbe, 5x conc. (Vial 1), mix gently, and store on ice.

4. In a 1.5 ml reaction tube on ice, prepare the PCR Mix per 20 µl or 100 µl reaction by adding the following components in the order mentioned below, then mix gently up and down.

Reagent	Volume [µl]	Final conc. [µl]
Water, PCR Grade (Vial 2)	9	–
Primer/Probes, 10x conc.	2	10
Master Mix, 5x conc. (Vial 1)	4	20
Total Volume	**15**	**30**

ⓘ *To prepare the PCR mix for more than one reaction, multiply the amount in the "Volume" column above by the number of reactions to be run plus 1 one additional reaction.*

5. – **For a 20 µl reaction:** Pipette 15 µl PCR mix into each precooled LightCycler® Capillary and add 5 µl of the DNA template.
– **For a 100 µl reaction:** Pipette 30 µl PCR mix into each pre-cooled LightCycler® Capillary and add 70 µl of the DNA template.

6. Seal each capillary with a stopper.
– If a LC Carousel Centrifuge is available, spin the capillaries in the LC Carousel Centrifuge.
– Alternatively, place the capillaries into cooled adpaters in a standard benchtop microcentrifuge, centrifuge at 700 × *g* (3,000 rpm) for 5 seconds, and transfer the capillaries into the LightCycler® Sample Carousel.

⚠ ***Place the centrifuge adapters in a balanced arrangement within the centrifuge.***

– Place the LightCycler® Sample Carousel into the LightCycler® Carousel-Based System and start the programmed protocol.

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) into amplification products, and the pretreatment of all successive PCR mixtures with UNG. If there are such amplicons in the PCR mixture, UNG cleaves the DNA at any site where a deoxyuridylate residue has been incorporated. Subsequently, the resulting abasic sites are hydrolyzed by high temperatures during the initial denaturation step, and cannot serve as PCR templates. Normal DNA contains thymidine, but no uridine, and is therefore not affected by this procedure.

⚠ ***When using LightCycler® FastStart DNA Master^{PLUS} HybProbe, perform prevention of carryover contamination with LightCycler® Uracil-DNA Glycosylase* prior to beginning real-time PCR. Proceed as described in the Instructions for use.***

ⓘ *The use of UNG might lower the melting temperature (Tm) in melting curve analysis by up to 1°C.*

3. Results

Quantification Analysis

The following amplification curves were obtained using the LightCycler® FastStart DNA Master^{PLUS} HybProbe in combination with the LightCycler® Control Kit DNA using the LightCycler® Red 640-labeled probe mixture and dilutions of the contained human genomic DNA in 1:10 steps as template.

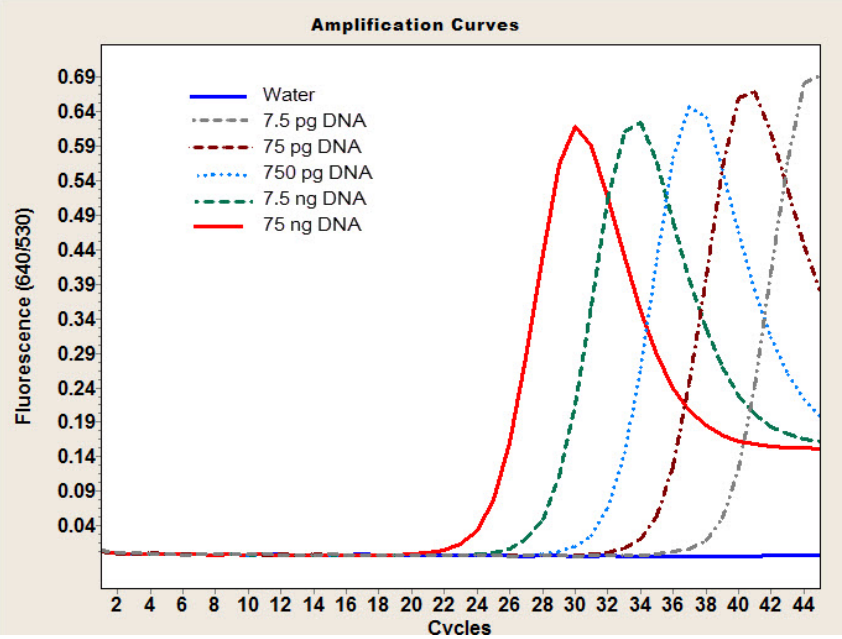

Fig. 1: Amplification curves in the quantification module of the LightCycler® Software 4.1.

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Starting amount of nucleic acid is very high.	Stop the program by clicking the **End Program** button. The next cycle program will start automatically.
	Number of cycles is too high.	Reduce the number of cycles in the protocol.
Log-linear phase of amplification just starts as the amplification program finishes.	Starting amount of nucleic acid is very low.	Improve PCR conditions (*e.g.,* $MgCl_2$ concentration, primer, and probe design).
		Use more starting material.
		Repeat the run.
	Number of cycles is too low.	Increase the number of cycles in the protocol.
		Use the **+10 cycles** button to increase the number of cycles in the program.
No amplification occurs.	Using wrong channel to display amplification on screen.	Change the channel setting on the programming screen. The data obtained up to this point will be saved.
	The programmed annealing temperature is lower than the melting temperature of a detection probe. Thus, this probe cannot emit a signal.	Ensure the annealing temperature is sufficiently low for probe hybridization.
	Measurements do not occur.	Check the cycle programs. For HybProbe detection, choose “single” as the acquisition mode at the end of the annealing phase.
	FastStart Taq DNA polymerase is not fully activated.	Make sure PCR included a pre-incubation step at 95°C for 10 minutes.
		Make sure denaturation time during cycles is about 15 seconds.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Check for missing or defective dye.
	Amplicon length is >1 kb.	Do not use amplicons >1 kb. Optimal results are obtained with a product length less than 700 bp.
	Impure sample material inhibits reaction.	Dilute sample 1:10 and repeat the analysis.
		Repurify the nucleic acids to ensure removal of inhibitory agents.
	Difficult template (*e.g.,* unusual GC-rich sequence).	Repeat PCR under same conditions and add increasing amounts of DMSO (up to 10% of the final concentration).
		If performance is still not satisfying, optimize annealing temperature in combination with a titration of DMSO.
Fluorescence intensity varies.	PCR mix is still in the upper part of the capillary. Air bubble is trapped in the capillary tip.	Repeat capillary centrifugation step.
	Pipetting errors	When using HybProbe probes and single-color detection, pipetting errors can be diminished through interpreting the results by dividing through channel F1 (530).
	Skin oils or dirt are on the surface of the capillary tip.	Always wear gloves when handling the capillaries.

Observation	Possible cause	Recommendation
Fluorescence intensity is too low.	Low concentration or deterioration of dyes in reaction mixtures; dyes not stored properly.	Store the dye-containing reagents at −15 to −25°C, and keep protected from light.
		Avoid repeated freezing and thawing.
		After thawing, store the LightCycler® FastStart DNA Master^{PLUS} HybProbe Master Mix at +2 to +8°C for a maximum of 1 week and keep protected from light.
		Low HybProbe probe signals can be increased by using a two times higher concentration of the red fluorophore-labeled probe than of the fluorescein-labeled probe.
	Reaction conditions are not optimized leading to poor PCR efficiency.	Primer concentration should be between 0.2 and 1.0 µM.
		Check annealing temperature of primers.
		Check experimental protocol.
		Always run a positive control along with your samples.
Negative control samples give a positive signal.	Contamination	Remake all critical solutions.
		Pipette reagents on a clean bench.
		Close lid of the negative control reaction immediately after pipetting.
		Use LightCycler® UNG to eliminate carryover contamination.
High background	Fluorescence signals are very low, therefore the background seems relatively high.	Follow general optimization strategies for the LightCycler® Carousel-Based System PCR.
	HybProbe probe concentration is too high.	HybProbe probe concentration should be in the range of 0.2 to 0.4 µM.
	Insufficient quality of HybProbe probes.	Prepare a new solution of HybProbe probes.
Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture (*e.g.,* salt concentration).	Check purity of template solution.
		Reduce variations in parameters such as salt concentration, LightCycler® UNG, and program settings.
Amplification curve decreases in later cycles.	"Hook effect": Competition between binding of the HybProbe probes and re-annealing of the PCR product.	This does not affect the interpretation of the results. It can be avoided by performing an asymmetric PCR, favoring amplification of the DNA strand to which the HybProbe probes bind.
No precise melting peak can be identified.	HybProbe probes are not homogenous, or contain secondary structures.	Redesign HybProbe probes.
	Pseudogenes lead to multiple PCR products.	Check PCR products on an agarose gel.

5. Additional Information on this Product

5.1. Test Principle

HybProbe probes are two different short oligonucleotides that bind to an internal sequence of the amplified fragment during the annealing phase of the amplification cycle. One probe is labeled at the 5' end with a fluorophore dye (LightCycler® Red 610, LightCycler® Red 640, Cy5, or Cy5.5) and to avoid extension, modified at the 3' end by phosphorylation. The other probe is labeled at the 3' end with fluorescein. When hybridized to the template DNA, the two probes are close enough to allow fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, fluorescein (the donor fluorophore) is excited by the light source of the LightCycler® Instrument. Fluorescein transfers part of this excitation energy to the dye (the acceptor fluorophore). Then, the dye emits fluorescence, which is measured by the LightCycler® Instrument. HybProbe probes that contain different dye labels can be used separately (for single-color detection experiments) or combined (for dual-color detection experiments). Color compensation is not necessary for single-color detection experiments. However, if you are using HybProbe probes to perform dual-color experiments in a single capillary, you must also use a color compensation file. Color compensation may be applied either during or after a run on the LightCycler® Instrument.

LightCycler® Red 610 and Cy5 can be used on a LightCycler® 2.0 Instrument only.

How this Product Works

LightCycler® FastStart DNA Master^{PLUS} HybProbe is a ready-to-use reaction mix designed specifically for the HybProbe probes detection format using the LightCycler® Carousel-Based System. It is used to perform hot start PCR in glass capillaries. Hot start PCR has been shown to significantly improve the specificity and sensitivity of PCR (Chou, Q., et al., 1992; Kellogg, D.E., et al., 1994; Birch, D.E., et al., 1996), by minimizing the formation of nonspecific amplification products at the beginning of the reaction.

FastStart Taq DNA Polymerase is a modified form of thermostable recombinant Taq DNA polymerase that is inactive at room temperature and below. The enzyme is active only at high temperatures, where primers no longer bind nonspecifically. The enzyme is 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.

LightCycler® FastStart DNA Master^{PLUS} HybProbe provides convenience, excellent performance, reproducibility, and minimal contamination risk. All you have to supply is template DNA, PCR primers, and HybProbe probes.

The reaction mix in this kit is optimized for a single $MgCl_2$ concentration, which works with nearly all primer combinations. You do not need to adjust the $MgCl_2$ concentration to amplify different sequences.

In combination with the LightCycler® Carousel-Based System and suitable PCR primers, this kit allows very sensitive detection and quantification of defined DNA sequences. Various sources of DNA (cDNA, genomic DNA, plasmid DNA, etc.) can be used. The kit is also suitable for SNP and mutation detection using melting curve analysis. Furthermore, the kit can be used to perform two-step RT-PCR in combination with a reverse transcription kit for cDNA synthesis.

⚠ ***The amplicon size should not exceed 1 kb in length. For optimal results, select a product length less than 700 bp.***

LightCycler® FastStart DNA Master^{PLUS} HybProbe offers convenience and ease of use because adaption of $MgCl_2$ in the reaction mixture is not necessary, thus avoiding time-consuming optimization steps. The new buffer formulation results in increased PCR robustness.

5.2. 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
- 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

5.3. Quality Control

The LightCycler® FastStart DNA Master^{PLUS} HybProbe is function tested using the LightCycler® Carousel-Based System.

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Roche 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 homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® Software 4.1	1 software package	04 898 915 001
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
Consumables		
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
Reagents , kits		
LightCycler® Control Kit DNA	1 kit, 50 reactions with 20 µl final volume each	12 158 833 001
LightCycler® FastStart DNA Master HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 003 248 001
	1 kit, 480 reactions of 20 µl final volume each	12 239 272 001
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
LightCycler® Color Compensation Set	1 set, 4 vials, 5 calibration runs	12 158 850 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
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

6.4. Trademarks

FASTSTART, HIGH PURE, HYBPROBE, LIGHTCYCLER and MAGNA PURE are trademarks of Roche.
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

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

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany