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LightCycler® 1536 DNA Probes Master

Version 04

Content version: January 2013

Ready-to-use hot start reaction mix for PCR with the LightCycler® 1536 System or the LightCycler® 480 System

Cat. No. 05 502 381 001

5 ml PCR Master Mix 5× conc.
(12,500 reactions 2 µl each, or
1,250 reactions 20 µl each)

Store the kit at −15 to −25°C
Protect vials 1 and 2 from light!

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Table of Contents

1. What this Product Does

Number of Tests

The kit is designed for

- 12,500 reactions with a final reaction volume of 2 µl each with a LightCycler® 1536 System,
- or 1,250 reactions with a final reaction volume of 20 µl each with a LightCycler® 480 System.

Kit Contents

Vial/Cap	Label	Contents/Function
1 colorless cap	Master Mix, 5× conc.	• 5 vials, 1 ml each, • 5× conc. ready-to-use hot start PCR Master Mix, • contains Taq DNA Polymerase, reaction buffer, dNTP mix, (with dUTP instead of dTTP), 16 mM $MgCl_2$, and a special dye for pipetting control
2 purple cap	Setup Control, 20× conc.	• 2 vials, 0.625 ml each, • for pipetting control of a further component of the PCR mixture in combination with the Master Mix
3 colorless cap	Water, PCR-grade	• 1 vial, 25 ml, to adjust the final reaction volume

Storage and Stability

The kit is shipped at room temperature.

Store the kit components as follows:

- Store at −15 to −25°C until the expiration date printed on the box.
- The kit can be stored for up to 4 weeks at +2 to +8°C, or for up to 1 week at room temperature (+15 to +25°C).
- Avoid repeated freezing and thawing.
- Protect from light.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform PCR with the LightCycler® 1536 DNA Probes Master include:

- LightCycler® 480 Instrument* or LightCycler® 1536 Instrument*
- LightCycler® 480 Multiwell Plate 96* or LightCycler® 480 Multiwell Plate 384* or LightCycler® 1536 Multiwell Plate*
- Standard swing-bucket centrifuge containing a rotor for multiwell plates and suitable adaptors
- Uracil-DNA Glycosylase, heat-labile* (optional ‡)
- Sterile 1.5 ml reaction tubes for preparing master mixes and dilutions
- Pipettes with nuclease-free, aerosol-resistant disposable pipette tips
- Liquid handling instrument for setup of low volume PCR reaction mixtures in a 1536-well plate (optional for LightCycler® 1536 Instrument) and a heat sealing instrument for sealing of the plates.

‡ *for prevention of carryover contamination; see Related Procedures section for details.*

Application

The LightCycler®1536 DNA Probes Master is a ready-to-use hot start reaction mix designed for fast real-time PCR using the LightCycler® 1536 System or the LightCycler® 480 System, enabling convenient up- and down-scaling of reaction volumes (*i.e.,* 96-well format: 10 to 100 µl; 384-well format: 5 to 20 µl; 1536-well format: 0.5 to 2 µl). This kit may also be used with high performance on other PCR instruments.

In principle, the LightCycler®1536 DNA Probes Master can be used for specific amplification of any DNA or cDNA template. Primers should be designed for an amplicon length of not more than 1,000 bp, or better less than 500 bp for optimal results.

The kit is optimized for detection using hydrolysis probes, for example, Universal ProbeLibrary probes. Carryover contamination by amplicons can be prevented by addition of Uracil DNA Glycosylase, heat-labile to the Master Mix containing sample DNA/cDNA.

The RealTime ready DNA Probes Master is specially optimized for demanding applications in automated high throughput PCR workflows. These reagents are ideal for high precision pipetting using liquid handling robotics in the low-volume range, and offer an exceptional room temperature stability during long processing times in automated laboratory workflows. The complete reaction mixture including the DNA template, primers, and probes can be stored for more than 24 hours at room temperature with no influence on the results of the PCR started afterwards.

The 5× concentrated Master Mix without primers and probes can also be dried within the LightCycler® Multiwell Plate 1536, and reconstituted after storage without a significant loss of activity.

The Master Mix is 5× concentrated for maximum flexibility in the composition of the reaction mixtures.

Pipetting Control Concept

The LightCycler®1536 DNA Probes Master enables a quality control which can be integrated in an automated data evaluation, to check for the correct composition of the reaction mixtures on the multiwell PCR plate.

The Master Mix (Vial 1) contains a special dye which is measured by the LightCycler® 1536 Instrument when the option "Master Control" is selected in the run definition in the LightCycler® 1536 Software. The Setup Control reagent (Vial 2) can be added to a further component of the reaction mixture (*e.g.,* DNA sample or primer/probe mix), and the correct combination of the two components is checked by the instrument when the option "Setup Control" is selected in the software. Lack of reagent in a well of the PCR plate due to errors in the plate setup (*e.g.,* pipetting malfunction) results in a flag for this well in the result table for this run in the software.

Assay Time

The LightCycler®1536 DNA Probes Master can be used for fast PCR protocols with run times of less than 50 min using a LightCycler® 1536 System or a LightCycler® 480 System.

2. How to Use this Product

2.1 Before You Begin

Sample Material

Use any template DNA (*e.g.,* cDNA, genomic, or plasmid DNA) suitable for PCR, as long as it is sufficiently free of PCR inhibitors.

⚠ For reproducible isolation of nucleic acids use either:
- the MagNA Pure LC Instrument* or the MagNA Pure Compact Instrument* and a dedicated MagNA Pure 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 homepage, www.roche-applied-science.com.

⚠ When using unpurified cDNA from a reverse transcription reaction with a high background of RNA, oligonucleotides, and $MgCl_2$, the amount of cDNA solution should not exceed 20% of the final PCR mix.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (Vial 3). In case of a genomic DNA or other DNA contamination problem, this can be detected using a negative control.

Primers

Suitable concentrations of PCR primers range from 0.05 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.5 µM each.

🔍 The optimal primer concentration is the lowest concentration that still results in a high rate of amplicon yield with a low Cp and adequate fluorescence dynamics for a given target concentration.

Probes

Suitable concentrations of hydrolysis probes range from 0.05 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.2 µM each.

🔍 The optimal probe concentration is the lowest concentration that results in the lowest Cp and adequate fluorescence dynamics for a given target concentration.

For a 5´-nuclease digestible hybridzation complex, the T_m of the hydrolysis probe must be higher than the T_m of the primers.

$MgCl_2$

The Master Mix of this kit is optimized with a fixed concentration of $MgCl_2$, which works with nearly all primer combinations. There is no need for adjustment.

2.2 Procedure

LightCycler® Instrument Protocol

The following procedure works with both the LightCycler® 1536 System and the LightCycler® 480 System. Established protocols can be transferred between the two systems with no need for reoptimization, independent of the reaction volumes (*e.g.,* 20 µl in a 96-well plate to 1 µl in a 1,536-well plate)´.

⚠ Program the instrument before preparing the reaction mixes.

A PCR protocol that uses the LightCycler®1536 DNA Probes Master should contain the following programs:

- **Initial Denaturation** for thorough denaturation of the template DNA
- **Cycling** for amplification of the target DNA
- **Cooling** of the thermal block at the end of the protocol

For more details on how to program the experimental protocol, see the Operator´s Guide of the LightCycler® 1536 or 480 Instrument.

The following table shows a typical protocol for PCR using a LightCycler® 1536 System and the LightCycler®1536 DNA Probes Master.

Setup				
Detection Format		**Pipetting Control**		
Hydrolysis Probes		Setup Control [1)]		
Programs				
Program Name		**Cycles**		
Initial Denaturation		1		
Cycling		45 [2)]		
Cooling		1		
Temperature Targets				
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**	**Acquisitions [n/°C)]**
Initial Denaturation				
95	None	00:01:00	4.8	–
Amplification				
95	None	00:00:00	4.8	–
60 [3)]	Single	00:00:30 [4)]	2.5	–
Cooling				
40	None	00:00:10	2.5	-

[1] Choose 'Setup Control' if Master Mix and Setup Control are present in the reaction mixture. Choose 'Master Control' if the Setup control is not included in the mixture and only the Master Mix has to be checked. Choose 'None' if no check for any component is desired.

[2] 45 cycles are suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[3] Most of the available assays are designed for an annealing temperature of +60°C. If the T_m of the primers afford different settings, select an annealing temperature 5°C below the calculated primer T_m for initial experiments. For assay optimization, choose the highest annealing temperature which still results in a high yield of amplicon with a low Cp and adequate fluorescence dynamics.

[4] For short amplicons < 150 bp, 30 s annealing should be sufficient in most cases. Especially for longer amplicons, longer annealing times are recommended.

Setup of the PCR Reaction

Prepare an assay to analyze a number of DNA samples using the LightCycler® 1536 Instrument as follows:

❶	Thaw one vial each of Master Mix Setup Control, and PCR-grade water (Vials 1 to 3). To ensure recovery of all the contents, briefly spin vials 1 and 2 min a microcentrifuge before opening, and mix carefully by pipetting up and down.
❷	Prepare a 2× concentrated reaction mixture of the 5× concentrated Master Mix, and the specific PCR primers and probes.
❸	Prepare 2× concentrated sample dilutions of the DNA samples and the 20× concentrated Setup Control.
❹	Dispense equal amounts of reaction mixture and sample dilutions to the respective wells of the LightCycler® 1536 Multiwell Plate using liquid handling robotics (*e.g.,* 1 µl each of the two solutions for a reaction volume of 2 µl).
❺	Seal the multiwell plate with adequate sealing film.
❻	Place the multiwell plate in a standard swing-bucket centrifuge, containing a rotor for multiwell plates with suitable adaptors, balance it with a suitable counterweight (*e.g.,* another multiwell plate), and centrifuge for 2 min at 1,500 × *g*.
❼	Load multiwell plate into the LightCycler® 1536 Instrument.
❽	Start the PCR program.

⚠ The above described setup scheme serves as an example. Other schemes are possible, including using other volumes, or analyzing one DNA sample with a number of different primer/probe sets.

⚠ Do not touch the surface of the LightCycler® Multiwell Plate 1536 when handling it.

2.3 Related Procedures

Prevention of Carryover Contamination

Uracil DNA N-Glycosylase (UNG) can help prevent carryover contamination in PCR. The prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix, instead of dTTP, in this kit) into amplification products, and 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; dUTP-containing contaminants will not serve as a PCR template.

⚠ Because your target genomic DNA or cDNA template contains thymidine and not uridine, it will not be digested by UNG and is thus not affected by this procedure.

⚠ To avoid cross-contamination by amplicons in experiments with the LightCycler® 1536 DNA Probes Master, Uracil-DNA Glycosylase, heat-labile* should be added. Follow the instructions in the Instructions for Use for this enzyme.

2.4 Quality Control

The LightCycler®1536 DNA Probes Master is function tested using the LightCycler® 1536 System.

3. Results

The following results were obtained using the LightCycler®1536 DNA Probes Master on the LightCycler® 1536 Instrument with primers and an UPL probe specific for Parvo B19, and dilutions of template DNA.

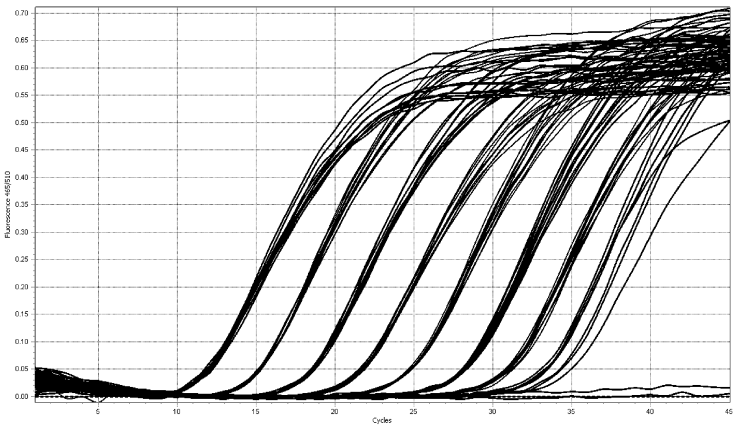

Fig. 1: Amplification curves obtained from dilutions of 10^8 to 1 target DNA copies per well. The steeply rising curves show a good reproducibility among replicates with the same target copy number and consistent distances between the different dilution steps down to 1 copy per well. PCR was performed in a reaction volume of 1 µl per well in a 1,536-well plate.

4. Troubleshooting

	Possible Cause	Recommendations
Log-linear phase of amplification just starts as the cycling program ends.	The number of cycles is too low.	• Increase the number of cycles in the cycling program. • Use more starting material. • Optimize PCR conditions (primer/probe design, protocol).
No amplification detectable	Wrong detection format was chosen for experimental protocol.	Select appropriate detection format for your assay and start again.
	Impure sample material inhibits reaction.	• Try a 1:10 dilution of your sample. • Purify the nucleic acids from your sample material to ensure removal of inhibitory agents.
	Amplicon length is >1 kb.	Do not design primers that produce amplicons >1 kb, which are inefficiently amplified. Optimal results are obtained with amplicons <500 bp.
	Difficult template, for example, unusual GC-rich sequence.	• Optimize temperatures and times used for the amplification cycles. • Optimize primer/probe sequences. • Repeat PCR but add increasing amounts of DMSO. (Use as much as 10% DMSO in the reaction.)
Fluorescence intensity varies.	Some of the reagent is still in the upper part of the microwell, or an air bubble is trapped in microwell.	Allow sufficient centrifugation time (*e.g.,* 2 min at 1,500 × *g*) for all reagent to reach the bottom of the microwell and/or to expel air bubbles.
	Skin oils or dirt on the surface of the microwell.	Always wear gloves when handling the Multiwell Plate.
Fluorescence intensity is very low.	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	• Keep dye-labeled reagents away from light. • Store the reagents at −15 to −25°C and avoid repeated freezing and thawing.
	Poor PCR efficiency (reaction conditions not optimized).	• Check concentrations of reagents and probes. • Optimize protocol.

	Possible Cause	Recommendations
	Chosen imaging time is too low.	• Choose adequate Roche Detection Format in combination with “dynamic” detection mode or • Increase imaging time when using “manual” detection mode. For details, see LightCycler® 1536 Instrument Operator’s Guide.
Negative control sample gives a positive signal.	Contamination	• Remake all critical solutions. • Pipet reagents on a clean bench. • Use UNG to eliminate carryover contamination.
High background	Fluorescence signals are very low, therefore the background seems relatively high.	Follow general strategies for optimizing PCR runs using the LightCycler® Real-Time PCR Systems Application Manual. To receive your personal copy, please contact your sales representative.
	Probe quality is poor.	Prepare a new probe solution.
High standard deviation of crossing point (Cp) values	Impure, heterogenous DNA template.	Use less amount of unpurified cDNA sample.
Unexpected bands on gel electrophoresis in the range of <50 bp	Additional bands due to reagent components.	These bands do not contribute to the final signal.

5. Supplementary Information

5.1 Conventions

5.1.1 Text Conventions

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

Text Convention	Usage
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.

5.1.2 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.

5.1.3 Changes to Previous Version

- Renaming of RealTime ready DNA Probes Master into LightCycler®1536 DNA Probes Master
- Updating Ordering Information
- Disclaimer of License updated.

5.2 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 homepage, www.roche-applied-science.com, and our Special Interest Sites for:

- Automated sample preparation (MagNA Lyser Instrument, MagNA Pure Compact System, MagNA Pure LC System, and MagNA Pure 96 System): http://www.magnapure.com
- Manual DNA and RNA preparation (High Pure Isolation Kits): http://www.roche-applied-science.com/napure
- Fast One-Step Cell Lysis (RealTime ready Cell Lysis Kit): http://www.gene-expression.roche.com
- LightCycler®1536 Real-Time PCR System: http://www.lightcycler1536.com
- RealTime ready assays: http://www.realtimeready.roche.com
- Universal ProbeLibrary probes: http://www.universalprobelibrary.com

	Product	Pack Size	Cat. No.
Instrument and Accessories	LightCycler® 1536 Instrument	1 instrument with control unit and accessories	05 334 276 001
	LightCycler® 1536 Software, Version 1.1	1 software package	06 569 382 001
	LightCycler® 1536 Multiwell Plate	10 × 10 plates	05 358 639 001
	LightCycler® 480 Instrument II	1 instrument (96 well) 1 instrument (384 well)	05 015 278 001 05 015 243 001
	LightCycler® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
	LightCycler® 480 Block Kit 384 Silver	1 block kit	05 015 197 001
	LightCycler® 480 Multiwell Plate 96, white	50 plates and foils	04 729 692 001
	LightCycler® 480 Multiwell Plate 384, white	50 plates and foils	04 729 749 001
	LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 1536 Kits for PCR	LightCycler® 1536 DNA Green Master	1 kit (for up to 12,500 reactions, 2 µl each)	05 573 092 001

	Product	Pack Size	Cat. No.
LightCycler® 480 Kits for PCR	LightCycler® 480 SYBR Green I Master	1 kit (5 × 100 reactions, 20 µl each)	04 707 516 001
		1 kit (10 × 500 reactions, 20 µl each)	04 887 352 001
	LightCycler® 480 Probes Master	1 kit (5 × 100 reactions, 20 µl each)	04 707 494 001
		1 kit (10 × 500 reactions, 20 µl each)	04 887 301 001
		1 kit (5000 reactions, 20 µl each)	04 902 343 001
	LightCycler® 480 Genotyping Master	1 kit (4 × 96 reactions, 20 µl each)	04 707 524 001
	LightCycler® 480 Control Kit	3 runs	04 710 924 001
Associated Kits and Reagents	Uracil-DNA Glycosylase, heat-labile	100 U	11 775 367 001
		500 U	11 775 375 001
	Transcriptor Reverse Transcriptase	250 U (for up to 25 reactions)	03 531 317 001
		500 U (for up to 50 reactions)	03 531 295 001
		2,000 U (for up to 200 reactions)	03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit (for up to 50 reactions, including 10 control reactions)	04 379 012 001
	First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit (for 30 reactions, including 5 control reactions)	11 483 188 001
	Transcriptor Universal cDNA Master	100 reactions	05 893 151 001

5.3 Disclaimer of License

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