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LightCycler® 1536 DNA Green Master

Version 02

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Easy-to-use hot start master mix for real-time PCR with the LightCycler® 1536 System

Cat. No. 05 573 092 001

5 ml PCR Master Mix 5× conc.
2 ml BrightGreen Dye 20× conc.
(12,500 reactions, 2 µl each)

Store the kit at −15 to −25°C
Protect vials 1, 2, and 4 from light!

www.roche-applied-science.com

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 Real-Time PCR 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 for adjusting the final reaction volume
4 Green Cap	BrightGreen Dye, 20× conc.	▪ 2 vials, 1 ml each ▪ 20× conc. fluorescent dye for PCR product detection

Storage and Stability

The kit is shipped on dry ice.
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 equipment and reagents required to perform real-time PCR with the LightCycler® 1536 DNA Green Master include:

- LightCycler® 1536 Instrument*
- LightCycler® 1536 Multiwell Plate*
- Heat sealing film / heat sealing instrument
- 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.

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

Application

The LightCycler® 1536 DNA Green Master is an easy-to-use hot-start master mix designed for fast real-time PCR applicatons using the LightCycler® 1536 System.

The LightCycler® 1536 DNA Green Master can be used for specific amplification of any DNA or cDNA template. For optimal results, primers should be designed for an amplicon length of no more than 500 bp, although good results are still possible with amplicons up to 1,000 bp in length.

Carryover contamination by amplicons can be prevented by adding Uracil-DNA Glycosylase, heat-labile to the Master Mix containing sample DNA/cDNA.

The LightCycler® 1536 DNA Green Master is specially optimized for demanding applications in automated high-throughput PCR workflows. The kit is ideal for precision pipetting using liquid handling robotics in the low-volume range, and offers exceptional room-temperature stability during long processing times in automated laboratory workflows.

The complete reaction mixture, including the DNA template and primers, can be stored up to 24 hours at room temperature (+15 to +25°C) with no influence on the results of the subsequent PCR.

For maximum flexibility, this Master Mix is 5× concentrated.

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

Pipetting Control Concept

The LightCycler® 1536 DNA Green Master features two dedicated controls (Master and Setup Control), enabling quality control of reaction mix setup when using LightCycler® 1536 Multiwell Plates in automated PCR workflows.

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 mix), whereby the following proper pipetting of that component is checked by the instrument when the option “Setup Control” is selected in the software.

Errors in the automated PCR setup workflow can be flagged in the result table of the LightCycler® 1536 Software for the pipetting steps of the Master Mix (“Master Control”) and the second individually chosen component of the PCR reaction mix (“Setup Contol”).

Assay Time

The LightCycler® 1536 DNA Green Master can be used for fast PCR protocols with run times of less than 50 minutes using a LightCycler® 1536 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 real-time PCR, as long as it is sufficiently free of PCR inhibitors.

⚠ For reproducible isolation of nucleic acids use:

- the MagNA Pure 96 Instrument, 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 visit 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 reaction mix.

Negative Control

Always run a negative control with the samples. To identify primer-dimers and contamination in assay components, prepare a negative control by replacing the template DNA with PCR-grade Water (vial 3).

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.

An appropriate primer design is a prerequisite for specific PCR amplification.

$MgCl_2$

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® 1536 Instrument Protocol

The following procedure works with the LightCycler® 1536 System.

⚠ Program the instrument before preparing the reaction mixes.

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

- **Initial Denaturation** for thorough denaturation of the template DNA
- **Cycling** for amplification of the target DNA
- **Melting Curve** for PCR product identification
- **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 for the LightCycler® 1536 Instrument.

The following table shows a typical protocol for real-time PCR using a LightCycler® 1536 System and the LightCycler® 1536 DNA Green Master.

Setup				
Detection Format		**Pipetting Control**		
Green Intercalating Dye		Setup Control [1]		
Programs				
Program Name		**Cycles**		
Preincubation		1		
2 Step Amplification		45 [2]		
Melting		1		
Cooling		1		
Temperature Targets				
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**	**Acquisitions [n/°C)]**
Preincubation				
95	None	00:01:00	4.8	–
Amplification				
95	None	00:00:00	4.8	–
60 [3]	Single	00:00:30 [4]	2.5	–
Melting Curve				
95	None	00:00:05	4.8	–
65	None	00:01:00	2.5	–
97	Continuous	–	0.1	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 is 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. If the T_m of the primers is below 60°C, run an appropriate 3 step protocol for amplification. For assay optimization, choose the highest annealing temperature that still results in a high yield of amplicon with a low Cp and adequate fluorescence dynamics.

[4] For short amplicons $<$150 bp, 30 seconds of annealing/elongation should be sufficient in most cases. For longer amplicons, longer annealing/elongation 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, PCR-grade Water, and BrightGreen Dye (vials 1 to 4). To ensure recovery of all the contents, briefly spin vials 1, 2, and 4 in 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, 20x concentrated BrightGreen Dye, and the specific PCR primers.
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	Heat seal the multiwell plate with adequate heat sealing film.
❻	Place the multiwell plate in a standard swinging-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 minutes 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 the plate.

2.3 Related Procedures

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) can help prevent carryover contamination in PCR. The prevention technique involves incorporating deoxyuridine triphos-phate (dUTP, a component of the Master Mix in this kit) instead of dTTP 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 Green Master, Uracil-DNA Glycosylase, heat-labile* should be added. Follow the instructions in the package insert for this enzyme.

2.4 Quality Control

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

3. Results

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

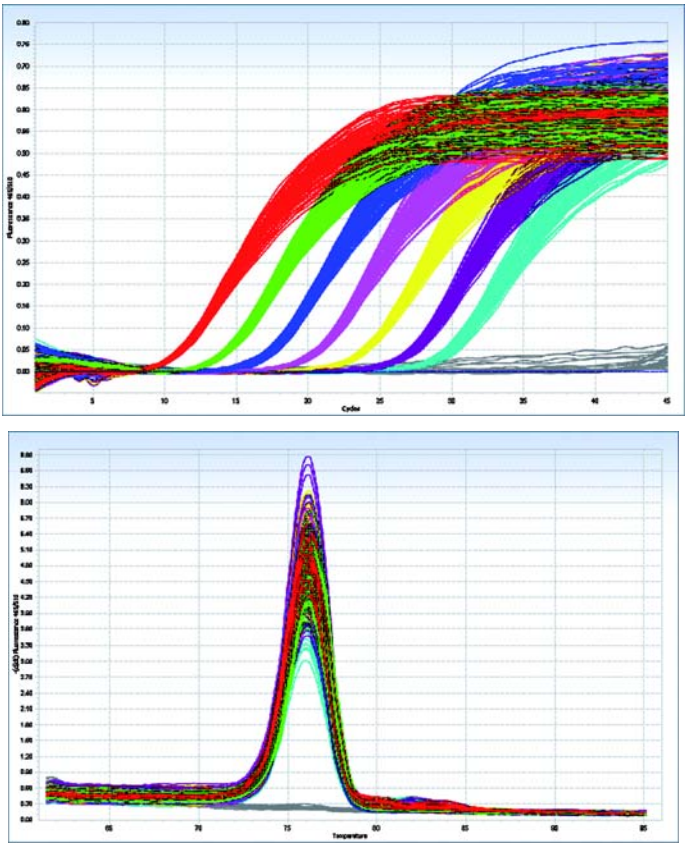

Fig. 1: Amplification curves obtained from 1:10 dilution series (10^7 to 10^1 target DNA copies per well) and no template control (NTC, grey); reaction volume 1 µl per well. The steeply rising curves show good reproducibility among replicate groups and dedicated distances (3.3 cycles) between the different dilution steps. The NTC represents the specificity of the PCR reaction by not showing primer-dimers, either in the amplification curve or in the melting curve analysis.

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 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 sequences. ▪ Repeat PCR using 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 the microwell.	Allow sufficient centrifugation time (*e.g.,* 2 minutes 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 reagent volumes. ▪ Optimize protocol. ▪ Always run a positive control with your samples.

	Possible Cause	Recommendations
Fluorescence Intensity is Very Low	Chosen imaging time is too low.	▪ Choose adequate Roche Detection Format in combination with “dynamic” detection mode. ▪ Increase imaging time when using “manual” detection mode. For details, see the LightCycler® 1536 Instrument Operator’s Guide
Negative Control Sample Gives a Positive Signal	Contamination, or presence of primer-dimers.	▪ Remake all critical solutions. ▪ Pipet reagents on a clean bench. ▪ Use UNG to eliminate carryover contamination. ▪ Re-design primer sequences.
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, contact your sales representative.
High Standard Deviation of Crossing Point (Cp) Values	Impure, heterogenous DNA template.	Use a smaller 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

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
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.

Symbols

In this Instruction Manual, 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.

5.2 Changes to Previous Version

Update of License Disclaimer

5.3 Ordering Information

Additional Products for Real-Time PCR

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, 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 1.0	1 software package	05 546 338 001
	LightCycler® 1536 Multiwell Plate	10 × 10 plates	05 358 639 001
Associated Kits and Reagents	Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 375 001 11 775 367 001
	Transcriptor Reverse Transcriptase	250 U 500 U 2,000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
	First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit	11 483 188 001
	Transcriptor Universal cDNA Master	100 reactions	05 893 151 001

5.4 Disclaimer of License

NOTICE: This product may be subject to certain use restrictions. Before using this product please refer to the Online Technical Support page (http://technical-support.roche.com) and search under the product number or the product name, whether this product is subject to a license disclaimer containing use restrictions.

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