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FastStart Essential DNA Green Master

Version 02

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Ready-to-use hot start reaction mix for real-time PCR using the LightCycler® Nano System

Cat. No. 06 402 712 001

5 × 1 ml
(5 × 100 reactions, 20 µl each)

Store the kit at −15 to −25°C

⚠ Keep FastStart Essential DNA Green Master away from light!

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PROTOCOL

1. What this Product Does

Number of Tests The kit is designed for 500 reactions with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Use	Content
1 green	FastStart Essential DNA Green Master; 2× conc.	▪ Ready-to-use hot start PCR mix ▪ Contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and $MgCl_2$	▪ 5 vials, 1 ml each (2× conc.)
2 colorless	FastStart Essential DNA Green Master; H_2O, PCR grade	To adjust the final reaction volume	▪ 5 vials, 1 ml each

Storage and Stability

Store the kit at -15 to -25°C until the expiration date printed on the label.

⚠ Keep the Master (Vial 1, green cap) away from light.

- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Label	Storage
FastStart Essential DNA Green Master; 2× conc.	▪ Store at -15 to -25°C. ▪ After first thawing, the master may be stored for up to 4 weeks at +2 to +8°C. ▪ **Avoid repeated freezing and thawing.** ▪ **Keep Vial 1 away from light.**
FastStart Essential DNA Green Master; H_2O, PCR grade	Store at -15 to -25°C.

The complete PCR mix (*i.e.,* FastStart Essential DNA Green Master supplemented with primers and template) is stable for up to 24 hours at room temperature. Keep the PCR mix away from light.

Additional Equipment and Reagents Required

Additional equipment and reagents required to perform reactions with the FastStart Essential DNA Green Master using the LightCycler® Nano System include:

- LightCycler® Nano Instrument*
- LightCycler® 8-Tube Strips (clear)*
- Standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptor
- LightCycler® Uracil-DNA Glycosylase* (optional ‡)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions

‡ For prevention of carryover contamination, see section Related Procedures for details.

* *available from Roche Applied Science*; see Ordering Information for details.

Application

The FastStart Essential DNA Green Master is designed for research studies. When used with the LightCycler® Nano System, this kit is ideally suited for hot start PCR applications. In combination with the LightCycler® Nano System and suitable PCR primers, this kit allows very sensitive detection and quantification of defined DNA sequences. The kit can also be used to perform two-step RT-PCR. It can also be used with heat-labile Uracil-DNA Glycosylase to prevent carryover contamination during PCR.

In principle, the kit can be used for the amplification and detection of any DNA or cDNA target. However, each amplification protocol will need to be adapted to the reaction conditions of the LightCycler® Nano Instrument and specific PCR primers will need to be designed for each target.

⚠ The amplicon size should not exceed 200 bp in length.

The ready-to-use FastStart Essential DNA Green Master offers convenience and ease of use because:

- no additional pipetting steps to combine enzyme and reaction buffer are necessary
- the addition of $MgCl_2$ to the reaction mixture is not necessary, thus avoiding time-consuming optimization steps

⚠ The performance of the kit described in this Instruction Manual is tested and guaranteed on the LightCycler® Nano System.

Assay Time

Variable, depending on the number of cycles and the annealing time.

For example, if the cycling program specifies 45 cycles and an annealing time of 10 seconds, elongation time of 15 seconds, a LightCycler® Nano PCR run will last about 53 minutes, including 10 minutes pre-incubation time, without melting curve.

2. How to Use this Product

2.1 Before You Begin

Sample Material

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors

⚠ For reproducible isolation of nucleic acids use:

- the MagNA Pure LC Instrument*, or the MagNA Pure Compact Instrument*, or the MagNA Pure 96 Instrument* together with a dedicated nucleic acid isolation kit (for automated isolation), or
- a High Pure Nucleic Acid Isolation Kit* (for manual isolation)
- RealTime ready Cell Lysis Kit* (for lysing cells prior to two-step real-time RT-PCR applications)

For details, see the Roche Applied Science Biochemicals catalog or home page, http://www.roche-applied-science.com.

- Use up to 50 – 100 ng complex genomic DNA or up to 10^8 copies plasmid DNA for a reaction volume of 20 µl. For larger volumes, the amount of template can be increased equivalently.

⚠ Using too much of template DNA might reduce the maximum fluorescence signal by outcompeting the SYBR Green I dye.

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 a maximum of 2 µl of that sample and applying a 10 minute pre-incubation at 95°C. This will result in lower crossing point (Cp), or quantification cycle (Cq) values with a decreased standard deviation.

Negative Control

Always run a negative control with the samples. To prepare negative controls:

- Replace the template DNA with PCR grade water (Vial 2; this will reveal whether a contamination problem exists).
- In a 2-step RT-PCR setup, omit addition of reverse transcriptase to the cDNA synthesis reaction (this will indicate whether DNA in RNA samples causes false-positive results).

Primers

Use PCR primers at a final concentration of 0.2 to 1 mM. The recommended starting concentration is 0.5 mM each.

The optimal primer concentration is the lowest concentration that results in the lowest Cq and an adequate fluorescence for a given target concentration.

$MgCl_2$

The composition of the FastStart Essential DNA Green Master is optimized for almost all primer combinations.

You do not need to add additional $MgCl_2$ to the mix to get efficient and specific PCR.

2.2 Experimental Protocol

LightCycler® Nano Instrument Protocol

The following procedure is optimized for use with the LightCycler® Nano Instrument.

Program the LightCycler® Nano Instrument before preparing the reaction mixes.

A LightCycler® Nano Instrument protocol that uses FastStart Essential DNA Green Master contains the following programs:

- **Pre-Incubation (Hold)** for activation of FastStart Taq DNA polymerase and denaturation of the DNA.
- **Amplification** of the target DNA.
- **Melting Curve** for PCR product identification.

For details on how to program the experimental protocol, see the LightCycler® Nano System Guides.

Experimental Protocol

The following table shows the PCR parameters that must be programmed for a LightCycler® Nano System PCR run with the FastStart Essential DNA Green Master using a LightCycler® 8-Tube Strip. The hold times shown here are a robust protocol (standard protocol) and can be shortened depending on your assay design[1].

Setup			
Run Settings			
Optics Settings			
Intercalating Dyes		Normal Quality	
Profile			
Programs			
Temp. (°C)	Ramp (°C/s)	Hold (s)	Acquire
Hold			
95	4	600[3]	
3-Step Amplification			
No. of Cycles: 45			
95	5	20[1] [4]	
60 primer dependent [2]	4	20[1] [4]	
72	4	20 [1] [4] [5]	✓
Melting			
65	4	60	
95	0.1	1	

[1] For well established assays (with amplicon size not exceeding 200 bp in length), you may shorten the amplification times to: 95°C for 10 seconds, 60°C for 10 seconds and 72°C for 10 - 20 seconds. 45 cycles are suitable for most assays. If the assay is optimized and has steep amplification curves and early crossing points (even when target concentrations are low), 40 cycles should be sufficient. Reducing the number of cycles will reduce the time required for the assay (fast protocol).

[2] For initial experiments, set the target temperature (the primer annealing temperature) 5°C below the calculated primer T_m.

[3] For some assays, a pre-incubation of 300 seconds is sufficient (fast protocol). However, if high polymerase activity is required in early cycles, a 600-second period is recommended, especially for higher reaction volumes and when working with unpurified cDNA samples as template. Do not use more than 2 µl unpurified cDNA sample.

[4] For greater precision in target quantification experiments, it can be advantageous (in some cases) to choose longer annealing and extension times for the amplification cycles. This is especially recommended for higher reaction volumes.

[5] Calculate the hold time for the PCR elongation step by dividing the amplicon length by 10 (*e.g.,* a 150 bp amplicon requires 15 seconds elongation time). Do not exceed the hold time for elongation below 10 seconds.

Preparation of the PCR Mix

Follow the procedure below to prepare one 20 µl standard reaction.

⚠ Always wear gloves during handling.

1
- Thaw one vial of "FastStart Essential DNA Green Master" (Vial 1, green cap) and Water, PCR grade (Vial 2, colorless cap).
- Briefly spin vials in a microcentrifuge before opening to ensure recovery of all the contents.
- Mix carefully by pipetting up and down and store on ice.

⚠ Keep the Master Mix away from light.

2 Prepare a 10× concentrated solution of the PCR primers.

3 In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 20 µl reaction by adding the following components in the order listened below:

Component	Volume
Water, PCR grade (Vial 2, colorless cap)	3 µl
PCR Primer, 10× conc.	2 µl
Master Mix, 2× conc. (Vial 1, green cap)	10 µl
Total Volume	**15 µl**

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 + sufficient additional reactions.

4
- Mix carefully by pipetting up and down. Do not vortex.
- Pipet 15 µl PCR mix into each reaction vessel of a LightCycler® 8-Tube Strip (clear).
- Add 5 µl of the DNA template.
- Close the reaction vessels.

❺	▪ For secure centrifugation, place the tube strips into a standard multi-well plate 96 (MWP) and balance them in the centrifuge. ▪ Centrifuge at 3,000 × *g* for a maximum of 30 seconds (in a standard swing-bucket centrifuge with suitable adaptor).
❻	Load the reaction vessels into the the LightCycler® Nano Instrument.
❼	Start the PCR program described above. ⚠ If you use reaction volumes different from 20 µl, it may be advantageous to adapt the hold times of all amplification steps (for example, 30 seconds for 100 µl).

2.3 Related Procedures

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.

⚠ Use only LightCycler® Uracil-DNA Glycosylase* in combination with the FastStart Essential DNA Green Master.

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

The use of UNG might influence the melting temperature (Tm) in melting curve analysis.

Two-Step RT-PCR

FastStart Essential DNA Green 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 LightCycler® Nano System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® Nano System procedure, using the cDNA as the starting sample material. The Transcriptor First Strand cDNA Synthesis Kit* is recommended for reverse transcription of RNA into cDNA. Synthesis of cDNA is performed according to the instructions provided with the kit.

⚠ For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount. If you use undiluted cDNA as template, we recommend a pre-incubation time of 10 minutes.

3. Results

Quantification Analysis

The following amplification curves were obtained using the FastStart Essential DNA Green Master, targeting mouse glyceraldehyde-3-phosphate dehydrogenase (Gapdh) mRNA. The intensity in relative fluorescence units (RFU) versus cycle number is displayed (see Figure 1).

For this well established and short (62 bp amplicon length) assay, a 2-step protocol (95°C 10 minutes; 40 x 95°C 10 seconds, 60°C 10 seconds) was used.

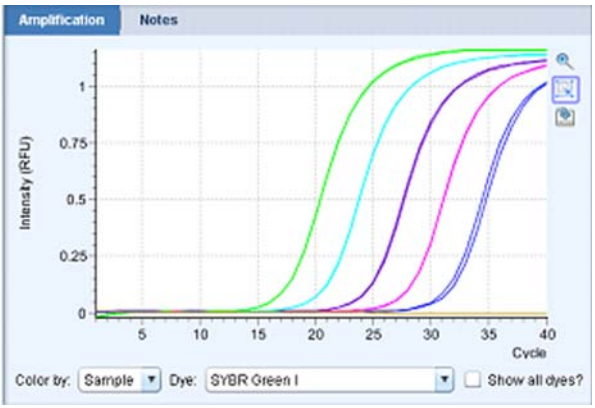

Fig. 1: Serially diluted samples containing cDNA derived from 50 ng, 5 ng, 500 pg, 50 pg, and 5 pg of total mouse RNA as starting template were amplified using the FastStart Essential DNA Green Master. As a negative control, template cDNA was replaced by PCR grade water.

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a melting curve analysis on the LightCycler® Nano Instrument. The resulting melting curves allow discrimination between primer-dimers and specific product (see Figure 2). The specific product melts at a higher temperature than the primer-dimers (no primer dimers visible for the Gapdh example shown below). The melting curves display the specific amplification of the Gapdh RNA when starting from cDNA derived from 50 ng, 5 ng, 500 pg, 50 pg, and 5 pg of total mouse RNA.

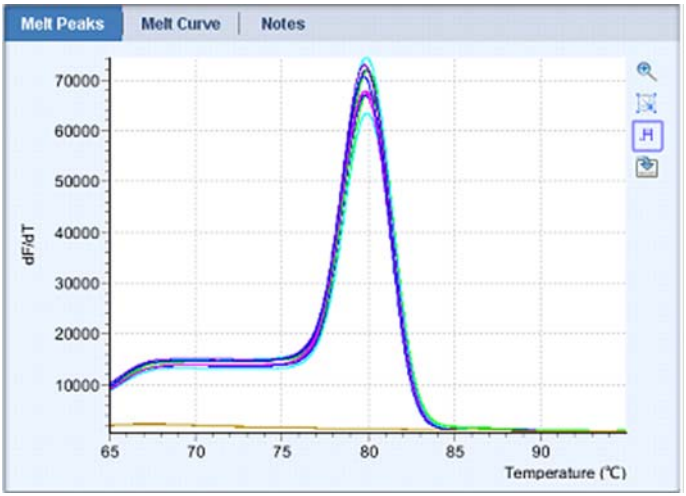

Fig. 2: Melting curve analysis of amplified samples with cDNA derived from 50 ng, 5 ng, 500 pg, 50 pg, and 5 pg of total mouse RNA as starting template. As a negative control, template DNA was replaced by PCR grade water.

4. Troubleshooting

	Cause	Recommendation
Log-linear phase of amplification just starts as the amplification program ends	Starting amount of nucleic acid is very low.	▪ Use more starting DNA template. ▪ Improve PCR conditions (*e.g.,* primer design). ▪ Repeat the run.
	Hold times in the amplification protocol are too short.	Optimize the run protocol by extending the hold times of annealing and elongation.
	The number of cycles is too low.	Increase the number of cycles in the amplification program.
No amplification detectable	Wrong detection format	Change the dye for the target under Samples - Targets.
	FastStart Taq DNA polymerase is not fully activated.	▪ Make sure PCR included a pre-incubation step at 95°C for 5 to 10 minutes. ▪ Make sure denaturation time during cycles is 10 seconds.
	Pipetting errors or omitted reagents.	Check for missing reagents. Check for missing or defective dye.
	Zoom function in the graph is active and only a small part of the chart is visible.	Undo the zoom function.
	Measurements do not occur.	Check the cycling program of the experimental protocol. For Intercalating Dyes, choose “Acquire” at the end of the elongation phase.
	Amplicon length is too long.	Do not use amplicons >200 bp.
	Impure sample material inhibits reaction.	▪ Do not use more than 5 µl of DNA per 20 µl PCR reaction mixture. ▪ Re-purify the nucleic acids to ensure removal of inhibitory agents.

	Cause	Recommendation
Fluorescence intensity is too low	Deterioration of dye in reaction mixtures; dyes not stored properly.	▪ Store the Master Mix at -15 to -25°C, and keep it away from light. ▪ Avoid repeated freezing and thawing.
	Wrong Optics Settings in the Run Settings folder.	Check the Optics Setting prior to each run.
	Reaction conditions are not optimized, leading to poor PCR efficiency.	▪ Primer concentration should be between 0.2 and 1.0 mM. ▪ Check annealing temperature of primers. ▪ Check experimental protocol. ▪ Always run a positive control along with your samples.
Fluorescence intensity varies	Varying volumes of master in different tubes.	Use one Master Mix for the different samples.
	Skin oils or dirt are present on the surface of the reaction vessel and/or lid.	Always wear gloves.
Amplification curve reaches plateau at a lower signal level than the other samples	Starting amount of genomic DNA is too high; DNA captures dye, producing a high background signal. There is not enough dye left to monitor the increase of fluorescence signal during amplification.	▪ Do not use more than 50 – 100 ng of complex genomic DNA in a 20 µl reaction. ▪ Instead of SYBR Green I, use a sequence-specific probe-based detection format (*e.g.,* hydrolysis probes) which allows analysis of up to 500 ng DNA (for a 20 µl reaction volume).
	Dye bleached.	Make sure the Master Mix is kept away from light. Avoid repeated freezing and thawing.
Negative control samples give a positive signal	Contamination, or presence of primer-dimers.	▪ Remake all critical solutions. ▪ Pipet reagents on a clean bench. ▪ Use heat-labile UNG to eliminate carryover contamination. ▪ Redesign primer sequences.
Double melting peak appears for one product	Two products of different length or GC-content are amplified (*e.g.,* due to pseudogenes or mispriming).	▪ Check products on an agarose gel. ▪ Elevate the reaction stringency by: ▪redesigning the primers ▪checking the annealing temperature ▪performing a “touch-down” PCR ▪using a probe-based detection format for better specificity

	Cause	Recommendation
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 heat-labile UNG, primer preparation, and program settings.
Only a primer-dimer peak appears, with no specific PCR product peak seen; or very high primer-dimer peaks	Sequence of primers is inappropriate.	Redesign primers.
	Quality of primer is poor.	Purify primer more thoroughly.
Primer-dimer and product peaks are very close together	Unusually high GC-content of the primers.	▪ Redesign primers. ▪ Run melting curve with lower ramp rate as in the High Resolution Melting with a ramp of 0.05°C/s.
Very broad primer-dimer peak with multiple peaks	Heterogeneous primers with primer-dimer variations (*e.g.,* concatemers, loops).	Redesign primers.
One peak of the same height occurs in all samples	Contamination in all samples.	Use fresh solutions.
High standard deviation of crossing point (Cp) values	Impure, heterogenous DNA template	▪ Increase pre-incubation time to 10 minutes. ▪ Use a maximum of 2 µl unpurified cDNA sample.
Baseline drift	Tubes not sealed properly.	Be sure to seal the tubes correctly.
	Cycler is loaded unbalanced.	If you use only two strips, be sure to load the 8-well strips balanced in the outer rows A and D.

5. Additional Information on this Product

How this Product Works

FastStart Essential DNA Green Master is a ready-to-use reaction mix designed specifically for applying the SYBR Green I detection format on the LightCycler® Nano Instrument. It is used to perform hot start PCR in LightCycler® 8-Tube Strips, or single tubes. Hot start PCR has been shown to significantly improve the specificity and sensitivity of PCR (1, 2, 3, 4) by minimizing the formation of non-specific amplification products at the beginning of the reaction.

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, 5 – 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

Test Principle

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I dye intercalates into the DNA helix (5). 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. Since SYBR Green I dye is very stable (only 6% of the activity is lost during 30 amplification cycles) and the LightCycler® Nano Instrument's optics match the wavelengths of excitation and emission, it is the reagent of choice when measuring total DNA.

The basic steps of DNA detection by SYBR Green I during real-time PCR on the LightCycler® Nano System 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 bind to the double strand. DNA binding results in a dramatic increase of the SYBR Green I molecules' light emission 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.

To prove that only your desired PCR product has been amplified, you may perform a melting curve analysis after PCR. In melting curve analysis, the reaction mixture is slowly heated to 95°C, which causes melting of double-stranded DNA and a corresponding decrease of SYBR Green I fluorescence. The instrument continuously monitors this fluorescence decrease and displays it as melting peaks. Each melting peak represents the characteristic melting temperature (T_m) of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). The most important factors that determine the T_m of dsDNA are the length and the GC-content of that fragment. If PCR generated only one amplicon, melting curve analysis will show only one melting peak. If primer-dimers or other non-specific products are present, they will be shown as additional melting peaks. Checking the T_m of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

References

1 Chou, Q *et al.*(1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acid Res.* **20** (7),1717-1723.

2 Kellogg, DE *et al.* (1994) TaqStart Antibody: hot-start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *BioTechniques* **16** (6),1134-1137.

3 Birch, DE *et al.* (1996) Simplified hot start PCR. *Nature* **381** (6581), 445-446.

4 Roche Diagnostics (1999) *PCR Manual*, 2nd edition. pp 52-58.

5 Zipper, H *et al.* (2004) Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. *Nuc. Acid Res.* **32** (12), e103.

Quality Control

The FastStart Essential DNA Green Master is function tested using the LightCycler® Nano Instrument.

6. Supplementary Information

6.1 Conventions

Text Conventions

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 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.

6.2 Changes to Previous Version

Disclaimer of License updated

6.3 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, visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites for:

- Real-Time PCR Systems (LightCycler® Carousel-based System, LightCycler® Nano System, LightCycler® 480 System, LightCycler® 1536 System, Universal ProbeLibrary, and RealTime ready): http://www.lightcycler.com
- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System, MagNA Pure LC, and MagNA Pure 96 System): http://www.magnapure.com
- DNA & RNA Preparation - Versatile Tools for Nucleic Acid Purification: http://www.roche-applied-science.com/napure
- RealTime ready Cell Lysis Kit: http://www.gene-expression.roche.com
- RealTime ready qPCR Assays with pre-tested UPL-probes: http://www.realtimeready.roche.com

	Product	Pack Size	Cat. No.
Instrument and Software	LightCycler® Nano Instrument	1 instrument (including USB stick with software)	06 407 773 001
Accessories	LightCycler® 8-Tube Strips (clear)	120 strips – including caps	06 327 672 001
PCR Reagents	FastStart Essential DNA Green Master	1 kit (5 × 100 reactions, 20 µl each)	06 402 712 001
	FastStart Essential DNA Probes Master	1 kit (5 × 100 reactions, 20 µl each)	06 402 682 001
Starter Packs	LightCycler® Nano DNA Green Starter Pack	LightCycler® Nano Instrument, 8 packs of Fast Start Essential DNA Green Master, 4 packs of strips	06 444 199 001
	LightCycler® Nano DNA Probes Starter Pack	LightCycler® Nano Instrument, 8 packs of Fast Start Essential DNA Probes Master, 4 packs of strips	06 444 202 001
Value Packs	LightCycler® Nano DNA Green Value Pack S	2 packs of Fast Start Essential DNA Green Master, 1 pack of strips	06 444 229 001

	LightCycler® Nano DNA Green Value Pack L	4 packs of Fast Start Essential DNA Green Master, 2 packs of strips	06 444 172 001
	LightCycler® Nano DNA Probes Value Pack S	2 packs of Fast Start Essential DNA Probes Master, 1 pack of strips	06 444 164 001
	LightCycler® Nano DNA Probes Value Pack L	4 packs of Fast Start Essential DNA Probes Master, 2 packs of strips	06 444 156 001
Associated Kits and Reagents	LightCycler® 480 RNA Master Hydrolysis Probe	1 kit (5 × 100 reactions, 20 µl each)	04 991 885 001
	LightCycler® Uracil-DNA Glycosylase	100 U (50 ml)	03 539 806 001
	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

6.4 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.

6.5 Trademarks

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SYBR is a trademark of Molecular Probes, Inc., Eugene, OR, USA.
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6.6 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

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- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

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