

LightCycler One-Step RT-PCR Systems: Choose the Right Kit for your RT-PCR Application



High-sensitivity methods for the detection and quantification of RNA targets are important tools of modern molecular biology. RNA cannot serve directly as a template for PCR. Prior to amplification it has to be transcribed into cDNA, which works as a template for polymerase during PCR. The combination of reverse transcription (RT) and PCR is called RT-PCR. Two major strategies are used for RT-PCR: one-step RT-PCR and two-step RT-PCR.

In a one-step RT-PCR both steps, cDNA synthesis and amplification, are performed in a combined reaction with the same target specific primers and within the same reaction tube. A one-step RT-PCR eliminates the need to open the reaction tube between the RT and PCR step. Major advantages of the one-step reaction are easy handling of the reactions with less hands-on time, less potential sources of contamination and, with the choice of the right enzymes, a maximum sensitivity. Most of the high throughput or routine RNA detections are run as one-step RT-PCR.

Roche Molecular Biochemicals is now offering two highly sensitive One-Step RT-PCR Systems for the LightCycler. Both systems are available for the SYBR Green I or the Hybridization Probe detection format.

- ▶ **New!** LightCycler-RNA Master Hybridization Probes
- ▶ **New!** LightCycler-RNA Master SYBR Green I
- ▶ LightCycler-RNA Amplification Kit Hybridization Probes (Improved!)
- ▶ LightCycler-RNA Amplification Kit SYBR Green I (Improved!)

Brief Overview

The new **LightCycler-RNA Master Kits** are ready to use "RNA Hot Start" reaction mixes including all components required (buffer, dNTPs and enzyme). The "RNA Hot Start" feature is achieved by using Tth DNA polymerase in combination with aptamers. Tth DNA polymerase is a thermostable enzyme with RNA-dependent reverse transcriptase activity and with DNA-dependent polymerase activity, allowing the combination of RT and PCR in a single tube reaction.

Aptamers are oligonucleotides, which bind to the active center of the polymerase and prevent attachment of

nucleic acid targets at temperatures below the optimal reaction temperature of the Tth enzyme. At higher temperatures the aptamers are released from the enzyme, and RT is initiated. "RNA Hot Start" with aptamers is highly effective and very convenient because it does not require additional incubation steps, pipetting steps or an extension of reaction time. The "RNA Hot Start" with aptamers does not interfere with other enzymatic processes, the online detection of amplification products or subsequent handling steps.

"RNA Hot Start" RT-PCR with the LightCycler-RNA Master Kits is becoming the standard for all applications that require maximum sensitivity, robustness and reliability (Fig. 1).

The improved **LightCycler-RNA Amplification Kits** are optimized one-step RT-PCR reagents including an enzyme mix and a reaction buffer. The enzyme mix contains AMV-RT for reverse transcription and Taq DNA polymerase for the PCR step. The reaction buffer mix includes all buffer components necessary, dNTPs as well as a basic level of $MgCl_2$. Optimizing the reagent buffer mix has been a major improvement to this kit (Fig. 2).

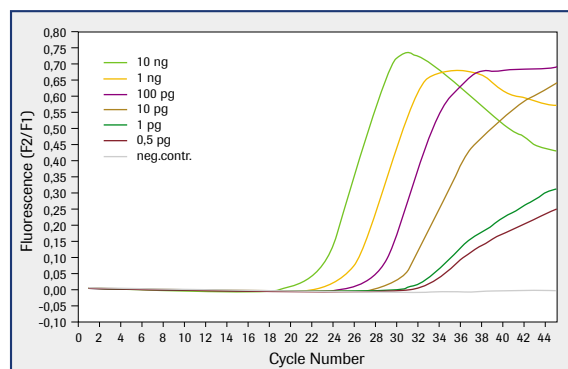


Figure 1: LightCycler-RNA Master Hybridization Probes. Serial dilutions of total liver RNA, detection of GA3PDH

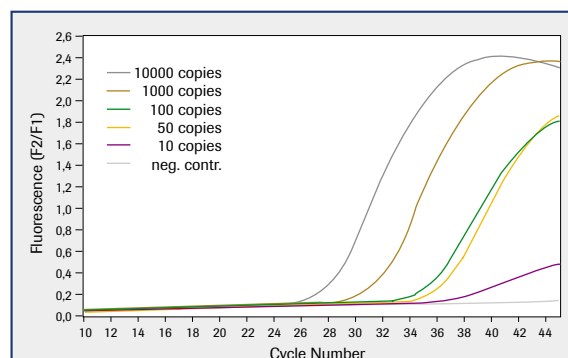


Figure 2: LightCycler-RNA Amplification Kit Hybridization Probes. Serial Dilutions of viral RNA (International Standard), detection of viral sequences

Figure 3:
SYBR Green I
detection format.
Sensitivity for
LightCycler One-
Step RT-PCR Kits

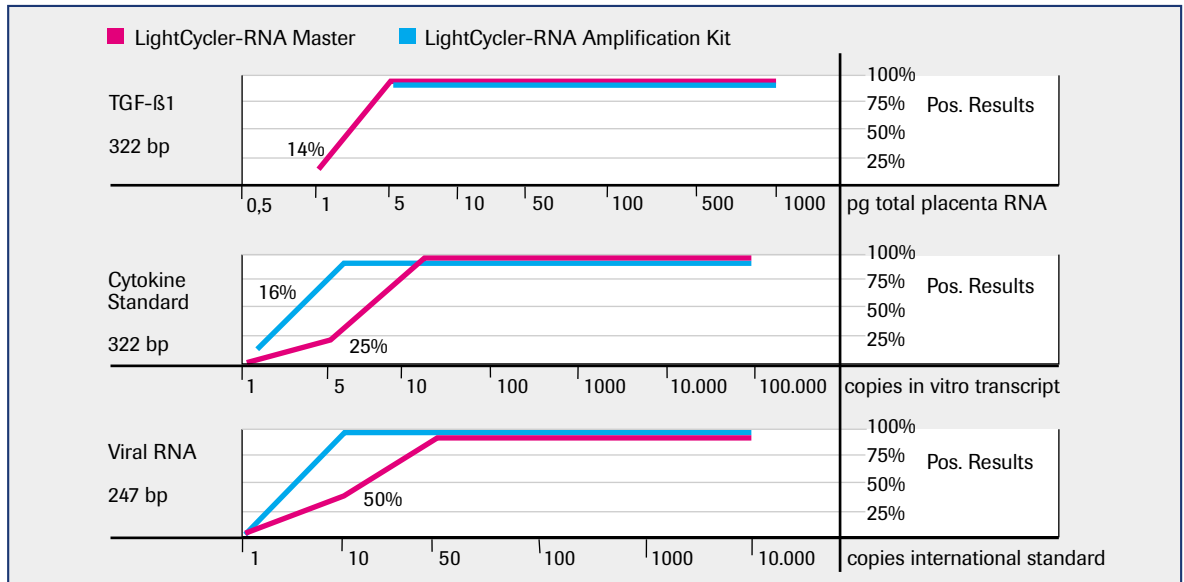
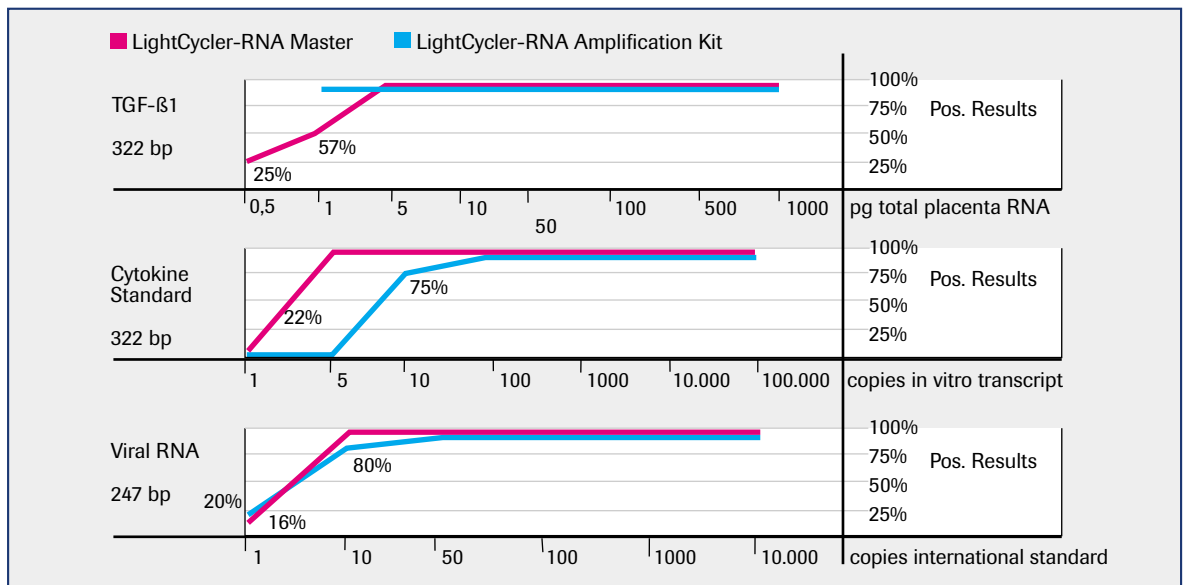


Figure 4:
Hybridization Probe
detection format.
Sensitivity for
LightCycler One-
Step RT-PCR Kits



A new Resolution Solution is included in the LightCycler-RNA Amplification Kit SYBR Green I to improve performance on templates with high secondary structure (e.g. GC rich templates).

One-step RT-PCR with the LightCycler-RNA Amplification Kits is ideally suited for all routine applications on standard RNA targets, where cost efficiency and reliability are needed.

Product Selection Guide for LightCycler One-Step RT-PCR Kits

Sensitivity

When highest sensitivity is required, the LightCycler-RNA Master Kits will be the first choice. In most cases these reagent systems will give outstanding sensitivities. Detection of viral RNA targets is one of the most

important applications of the LightCycler-RNA Master Kits. However, the LightCycler-RNA Amplification Kits will be more than an alternative. As shown for TGF-β1, these kits are able to deal with complex targets, and guarantee high sensitivities for these applications. Transcripts of various targets and expression levels are detected with total RNA amounts as low as 5 pg, reflecting 1/10th of the RNA content of a human cell (Fig. 3 and Fig. 4).

Robustness

Both systems are able to amplify templates with difficult sequences (e.g. secondary structures, GC rich sequences). The LightCycler-RNA Master Kits do not need any adjustments due to the high RT temperature. The "RNA Hot Start" also contributes to robustness by delivering a higher specificity in excluding unspecific elongation during the first heating phase.

Convenience

Using the LightCycler-RNA Master Kits is the most convenient way to perform one-step RT-PCR because all required reagents are mixed in one vial. Pipetting steps and contamination risks are reduced to an absolute minimum.

The LightCycler-RNA Master Kits display outstanding stability even at elevated temperatures. Pipetting of reactions in environments that are not strictly temperature controlled, e.g. on an automated pipettor, is possible. The hot start feature will support this setup since the Tth DNA polymerase is not active at room temperature.

Decontamination

In all LightCycler Kits, including the LightCycler One-Step RT-PCR Kits, decontamination with heat labile UNG is possible.

Reagent selection hints

When establishing a new LightCycler RT-PCR reaction, the LightCycler-RNA Master Kits are recommended for a first approach. These kits will ensure highest sensitivity for a broad range of targets, they are very robust and highly convenient. Together with "RNA Hot Start" they are the best choice for achieving maximum sensitivity for a broad range of targets (Table 1).

However, for some targets, as demonstrated on TGF- β 1, LightCycler-RNA Amplification Kits will give better results than the other systems.

You need...	We recommend...	
	LightCycler-RNA Master ^{NEW}	LightCycler-RNA Amplification Kit ^{Improved}
Highest Sensitivity	+++	++(+)*
"RNA Hot Start"	+++	-
Stability at elevated Temperatures	+++	-
Convenience	+++	++
Alternatives for complex targets	+++	++

Table 1: Reagent selection hints

*: similar sensitivities as with LightCycler-RNA Master Kits are observed after optimization, depending on the target.

<http://biochem.roche.com/lightcycler>



Product	Pack Size	Cat. No.
NEW! LightCycler-RNA Master SYBR Green I	1 Kit (96 reactions)	3 064 760
NEW! LightCycler-RNA Master Hybridization Probes	1 Kit (96 reactions)	3 018 954
LightCycler-RNA Amplification Kit SYBR Green I	1 Kit (96 reactions)	2 015 137
LightCycler-RNA Amplification Kit Hybridization Probes	1 Kit (96 reactions)	2 015 145

1000th LightCycler Awarded to Medical Center

Roche Molecular Biochemicals recently sold its 1000th LightCycler Instrument. To commemorate this milestone of LightCycler acceptance by the international research community, we decided to donate a LightCycler Instrument to a charitable research institution. Considered for the award were laboratories that would use the LightCycler System to benefit the lives of others, but that lacked sufficient funding to purchase the instrument.

Based on those criteria, Roche selected Dr. Zacharie Brahmi, Director of Immunology and HLA Lab at Riley Hospital for Children in Indianapolis, Indiana/USA. Riley Hospital is internationally renowned for its cutting-edge pediatric research. On November 21, members of Roche Molecular Biochemicals presented the donated LightCycler Instrument to Dr. Brahmi, who hopes to use it for genetic research, forensic analysis, and Mycoplasma detection.



Presentation of the 1000th LightCycler Instrument to Riley Hospital for Children. Shown from left to right are Nathalie Chiasson, Director of Sales, Roche Molecular Biochemicals; Dr. Zacharie Brahmi, Director of Immunology and HLA Lab at Riley Hospital for Children; Glenn Martin, Product Manager LightCycler System, Roche Molecular Biochemicals (USA); Dr. Juergen Flach, Senior Vice-President for Molecular Biochemicals; Wayne Burris, Chief Financial Officer for Roche Diagnostics Corporation.