Fast qRT-PCR with LightCycler® 480 RNA Master Hydrolysis Probes

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Introduction

Scientists who want to increase throughput in their workflows are interested in shortening the reaction times. Fast PCR can be achieved using a fast PCR instrument like the LightCycler® 480, and the appropriate reagent like the LightCycler® 480 RNA Master Hydrolysis Probe. The enzyme component allows fast reverse transcription and the included hot-start system does not require an extra heat treatment for chemical activation. Therefore, the first two reaction steps can be shortened compared with standard protocols. In order to explore the fastest conditions possible we analyzed several variables.

The LightCycler® 480 RNA Master Hydrolysis Probes is an easy-to-use hot-start reaction mix, specifically adapted for one-step RT-PCR under the rapid and accurate cycling conditions of the plate-based LightCycler® 480 Instruments using hydrolysis probes (*e.g.,* Universal ProbeLibrary probes) as detection format. The kit provides reagents, including an RNA master mix (with buffer, nucleotides, and enzyme), a $Mn(OAc)_2$ stock solution, PCR-grade water, and enhancer solution. The LightCycler® 480 RNA Master Hydrolysis Probes can be used in conjunction with heat-labile Uracil DNA Glycosylase for carryover prevention during PCR.

Materials and Methods

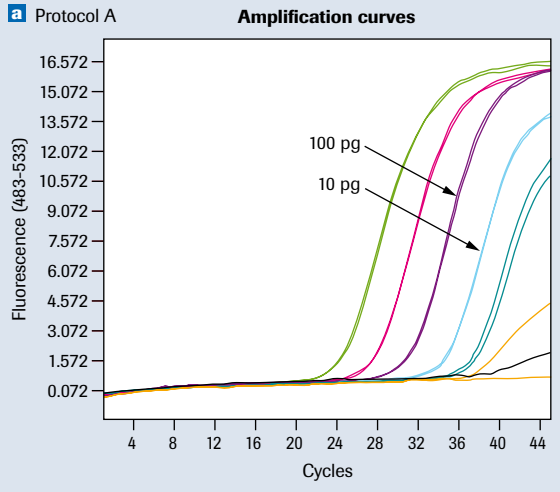
The experiments were performed with HeLa total RNA in dilutions from 10 ng to 0.1 pg (A, standard protocol), and 100 pg to 0.1 pg for protocols B to D. Primers directed to ABCC2 RNA were used and the products were detected with a Universal ProbeLibrary probe.

Results and Discussion

In protocol A we used a fast RT step and a conventional PCR protocol. In protocols B and C the RT step times were reduced. In protocol D the incubation times for the PCR cycles were drastically reduced. In order to check

Table 1: Amplification protocols tested.

Protocol	A	B	C	D	
Reverse transcription	3 min	10 sec	3 sec	3 sec	
Initial denaturation	30 sec	30 sec	30 sec	30 sec	
PCR	15 sec	15 sec	15 sec	1 sec	x 45
	60 sec	60 sec	60 sec	10 sec	
	1 sec	1 sec	1 sec		
Σ hold times	60 min 30 sec	57 min 40 sec	57 min 33 sec	9 min 33 sec	
	Cp values	**Cp values**	**Cp values**	**Cp values**	
100 pg total RNA	31.2	32.2	32.5	33.1	
	31.3	32.3	32.7	33.1	
10 pg total RNA	34.7	35.7	35.6	36.5	
	34.7	35.0	35.7	36.3	



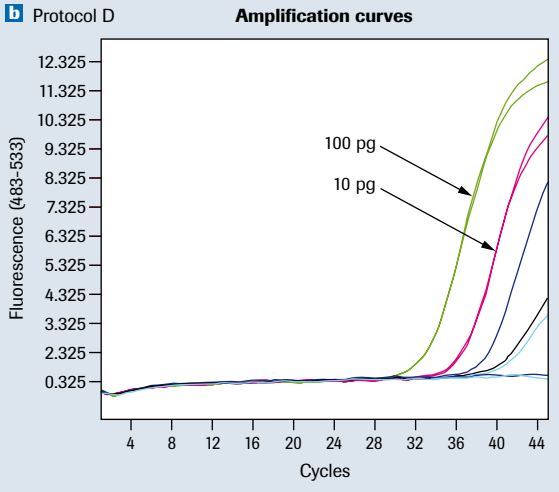


Figure 1: RNA amplification with the LightCycler® 480 RNA Master Hydrolisis Probes. RNA was amplified to the same extent using **(a)** standard amplification protocol A or **(b)** amplification protocol D.

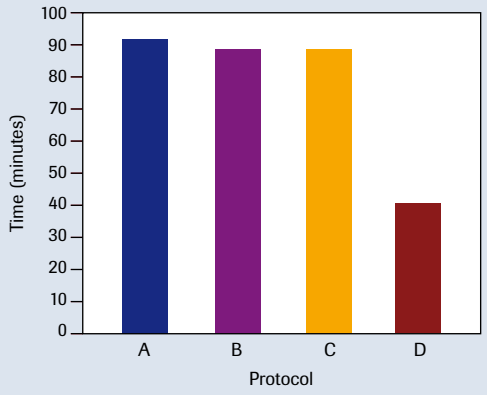


Figure 2: Impact of amplification protocol on total run time. The shortest run time was achieved with amplification protocol D.

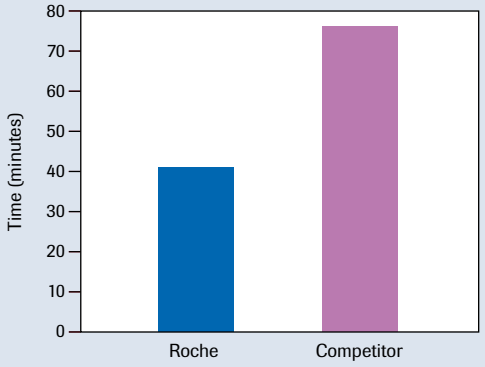


Figure 3: Comparison of overall reaction times using different qRT-PCR systems. Results can be achieved faster using the LightCycler® 480 RNA Master Hydrolysis Probes.

whether contaminating DNA was detected rather than a product derived from the RNA, a control reaction was performed with the same RNA, primer and probe but using LightCycler® 480 Fast DNA Master. This control reaction did not result in any significant amplification (not shown).

As shown in Table 1, the cp values depicted for the 100-pg and 10-pg template concentrations are quite similar. LightCycler® 480 RNA Master Hydrolysis Probes amplified the RNA to the same extent and similar sensitivity independent of the amplification protocol chosen (Figure 1 and Table 1). Thus, a very short total run time can be achieved and more than 55% time saved (Figure 2).

A comparison of the reaction times of protocol D with those recommended for a competitor product is depicted in Figure 3. The competitor system requires nearly twice the reaction time the LightCycler® 480 System requires. ■

Product	Pack Size	Cat. No.
LightCycler® 480 RNA Master Hydrolysis Probes	5x100 reactions	04 991 885 001