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Evaluation of RT-qPCR protocols with

Volcano3G® RT-PCR Probe 2x Master Mix

Medix Biochemica

Introduction

Volcano3G® RT-PCR Probe 2x Master Mix contains a unique highly thermostable *Taq*-derived DNA polymerase with reverse transcriptase activity and a *Taq* DNA Polymerase for efficient PCR amplification. Both enzymes use a hot-start aptamer-based technology along with an optimized master mix formulation enabling sensitive and reliable amplification.

Volcano3G® is best for versatile reverse transcription and RT-qPCR protocols. The master mix permits either true quantitative or rapid detection of RNA targets[1,2]. The intrinsic thermostability and reverse transcriptase activity of the enzyme permits direct[3] or more traditional RT-qPCR at a higher temperature range from 58°C to 70°C and higher.

Multiple options for RT-qPCR with Volcano3G® include a cycling, isothermal or touchdown reverse transcription step or even no reverse transcription step at all.

Methods

Volcano3G® RT-PCR Probe 2x Master Mix was used to amplify up to four different targets in singleplex reactions. The experiments were carried out in triplicate on whole cell lysates as template in a QuantStudio5 PCR cycler. Multiple RT-qPCR protocols were tested to differentiate the performances of the master mix for early (cycled RT-qPCR and touchdown PCR) or rapid (isothermal or zero-step RT-qPCR) detection. The four different protocols are as per the tables below:

A) Cycled RT-qPCR

Temperature	Time	Cycles
95°C	1 sec	10x
70°C	1 min	
95°C	5 sec	50x
66°C	30 sec	

B) Touchdown RT-qPCR

Temperature	Time	Cycles
95°C	1 sec	10x
70°C ➔ 66°C	1 min	
95°C	5 sec	50x
66°C	30 sec	

C) Isothermal RT-qPCR

Temperature	Time	Cycles
70°C	15 min	1x
95°C	5 sec	50x
66°C	30 sec	

D) Zero-step RT-qPCR

Temperature	Time	Cycles
95°C	20 sec	1x
95°C	5 sec	50x
66°C	30 sec	

Performing reverse transcription at a higher temperature can benefit assays dealing with complex samples (e.g. matrices, RNA secondary structures) and increase specificity.

Results

The corresponding results demonstrate that a cycled RT step or a touchdown RT-qPCR generate the earliest Cq values as in figure 2 and the amplification curves are similar for both types of experiments in figures 1A and 1B. A cycled RT step yields reliable cDNA synthesis and early detection using a conventional PCR cycler. Alternatively, touchdown PCR reduces the formation of primer-dimers and non-specific binding events. The use of Volcano3G® RT-PCR Probe 2x Master Mix and a touchdown 'RT step' ensures primary synthesis of cDNA complemented by the correct amplification of the target.

In comparison, the results from isothermal or zero-step RT-qPCR show delayed Cq as in figure 2 while the fluorescence signal remains like the previous protocols in figures 1C and 1D. However, the run time is overall shorter, this type of RT-qPCR is hence suitable for a more qualitative detection of RNA targets with a faster time to results.

Conclusions

- Volcano3G® RT-PCR Probe 2x Master Mix is a versatile master mix permitting multiple scenarios of RT-qPCR for quantitation or detection of RNA targets.
- The blend of enzymes in Volcano3G® RT-PCR Probe 2x Master Mix benefits quantitative detection of RNA targets with a cycled or touchdown RT step.
- The high thermostability of Volcano3G® RT-PCR Probe 2x Master Mix enables any RT step at a higher temperature within the suitable 58-70°C margin.

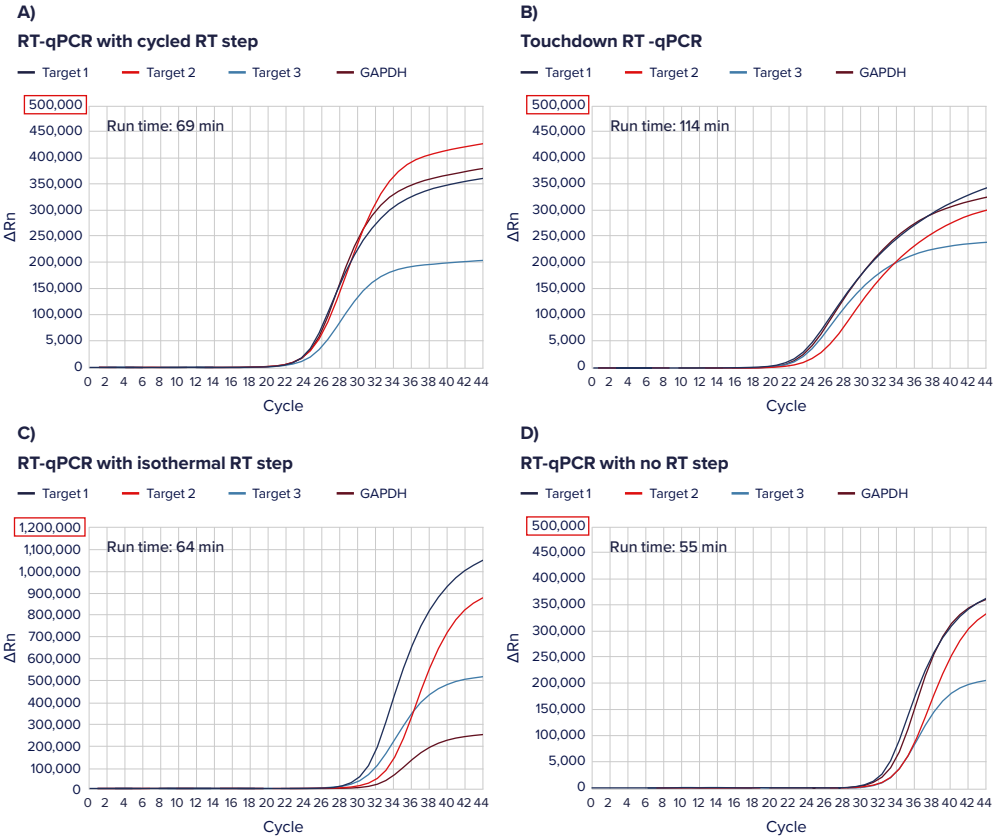

Figure 1: Results from different RT-qPCR protocols. A) Amplification from a RT-qPCR with cycled RT step ,B) Amplification from a RT-qPCR with a touchdown RT, C) Amplification from traditional isothermal RT-qPCR, D) Amplification from a zero-step RT-qPCR. Amplification curves are plotted as the mean of the experimental triplicate.

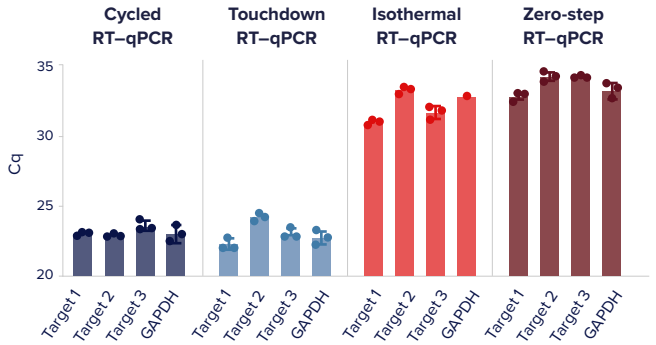

Figure 2: Cq values from all protocols plotted for each amplified RNA target. All experiments were performed in triplicate with cell lysates as template.

References:
1. Kuiper, J. W. P. et al. Detection of SARS-CoV-2 from raw patient samples by coupled high temperature reverse transcription and amplification. PLoS One 15, 1–13 (2020).
2. Schwaderer, J. et al. Pharmacological LRH-1/Nr5a2 inhibition limits pro-inflammatory cytokine production in macrophages and associated experimental hepatitis. Cell Death Dis. 11, (2020).
3. Chovancova, P., Merk, V., Marx, A., Leist, M. & Kranaster, R. Reverse-transcription quantitative PCR directly from cells without RNA extraction and without isothermal reverse-transcription: A 'zero-step' RT-qPCR protocol. Biol. Methods Protoc. 2, 1–6 (2017).

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