One-step NZYSpeedy RT-qPCR Green kit

Catalogue number:

MB34601, 100 reactions x 20 µL
MB34602, 500 reactions x 20 µL

Description

One-step NZYSpeedy RT-qPCR Green kit is an optimized and highly efficient reaction mixture developed for first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The kit includes a One-step NZYSpeedy qPCR Green master mix developed for fast PCR, provided as a 2× reaction mixture, which contains all components necessary for real-time PCR, including a green intercalating dye, dNTPs, stabilizers and enhancers. In addition, a separate NZYRT mix that comprises a balanced mixture of both Reverse transcriptase and Ribonuclease Inhibitor is also provided. This kit enables fast and highly reproducible procedures on the most common real-time PCR apparatus from either total RNA or mRNA. It is optimized for intercalating green dye detection on different instruments. The latest developments in PCR enhancers have been incorporated in the One-step NZYSpeedy RT-qPCR Green kit, including buffer chemistry and a polymerase with hot-start-like activity. This optimized mixture together with the NZYRT mix results in highly sensitive and successful results.

Shipping Conditions

The product is shipped with dry ice.

Storage Conditions

This master mix should be stored at -85°C to -15°C in a freezer without defrost cycles in order to guarantee maximal shelf life. Minimize the number of freeze-thaw cycles by storing in working aliquots. The product will remain stable till the expiry date if stored as specified. The green dye is light sensitive, as such the master mix should be protected from light whenever possible.

Compatible real-time PCR instruments

The kit does not contain any passive reference dye and thus it is compatible with real-time PCR instruments that do not require a passive reference signal for data normalization. It has been optimized to be compatible with the following real-time PCR instruments:

Bio-Rad®: CFX96™; CFX384™; Opticon™; Opticon™ 2

Qiagen (Corbett): Rotor-Gene™ 3000, 6000 & Q

Roche: Lightcycler® 96, 480 & Nano

Protocol

The following protocol serves as a general guideline and a starting point for any One-step RT-qPCR procedure. Optimal reaction conditions (incubation times and temperatures, template concentration) may vary and, in particular cases, may require further optimization.

RT-qPCR reaction set-up: the given volumes are based on a standard 20 µL final reaction mix and can be scale adjusted.

One-step NZYSpeedy qPCR Green master mix (2x)	10 µL	1×
10 µM forward primer	0.8 µL	400 nM
10 µM reverse primer	0.8 µL	400 nM
NZYRT mix	0.8 µL	-
Template	up to 7.6 µL	-
Nuclease-free water	up to 20 µL	-

Testing and Ct values: When comparing this RT-qPCR master mix with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed.

Suggested thermal cycling conditions

One-step NZYSpeedy RT-qPCR Green kit was optimized for the amplification of RNA fragments up to 200 bp under different RT-qPCR cycling conditions. The table below displays a standard 2-step cycling setup optimized on a number of platforms. However, these conditions may be adapted to suit different machine-specific protocols.

Cycles	Temp.	Time	Main Reaction
1	50 °C	10-20 min (*)	Reverse Transcription
1	95 °C	2-5 min	Polymerase activation
40	95 °C	5 sec	Denaturation
	60 °C	30 sec -50 sec	Annealing/Extension

(*) Reverse transcriptase has a high processivity and may take as little as 10 minutes to synthesize cDNA. However, in specific situations increasing reaction time up to 20 minutes may be beneficial.

General considerations

Because of the chemical instability of the RNA and the ubiquitous presence of RNases, working with RNA is more demanding than working with DNA. Therefore, special precautions should be taken when working with RNA. We recommend using RNase-free plasticware/reagents and work in an RNase-free area. In addition, to help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers: The specific amplification, yield and efficiency of any real-time PCR can be affected by both sequence and primers concentration, as well as by the fragment length. We strongly recommend taking the following suggestions into consideration when designing and running your RT-qPCR experiment:

- Primers should have a melting temperature (T_m) of approximately 60 °C;
- The fragment should be between 80-200 bp length and not superior to 400 bp;

- Final primer concentrations of 400 nM are suitable for most green-based reactions. However, to determine the optimal concentration we recommend titrating in the range 0.1-1 µM. The forward and reverse primers concentration should be equimolar;
- Where possible design intron spanning primers to avoid gDNA amplification.

Template: It is important that the RNA template is purified and devoid of any contaminating of RT-qPCR inhibitors (e.g. EDTA). The recommended amount of template is dependent upon the type of RNA used. Please consider the following points when selecting RNA templates:

- **Total RNA:** purified total RNA can be used in the range from 1 pg to 1 µg per 20 µL reaction;
- **mRNA:** purified mRNA can be used from 0.01 pg per 20 µL reaction.

To obtain high yield of highly purified RNA we suggest using the NZY Total RNA Isolation Kit (MB134).

$MgCl_2$: It is not necessary to supplement the reaction mixture with $MgCl_2$ as the One-step NZYSpeedy qPCR Green master mix (2x) already contains an optimized concentration of $MgCl_2$.

RT-qPCR controls: Data reliability may be affected by the presence of contaminating DNA. We suggest that you always include no-RT control reactions, by replacing the NZYRT mix with PCR-grade water. Furthermore, refer to the instrument instructions for the option of melt-profile analysis.

RT-qPCR optimization: the cycling conditions can be varied to suit different machine-specific protocols. It may be necessary to improve the efficiency of some reactions: the reverse transcription reaction time can be extended up to 30 minutes; the annealing/extension time can be extended up to 60 seconds and/or the temperature can be increased up to 65°C.

Quality control assays

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the kit mixes for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

One-step NZYSpeedy RT-qPCR Green kit is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

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