NZYSpeedy One-step RT-qPCR Probe Master Mix (2x)

Catalogue number:

MB40501, 2 mL (200 x 20 µL)
MB40502, 5 mL (500 x 20 µL)
MB40503, 20 mL (2000 x 20 µL)

Description

NZYSpeedy One-step RT-qPCR Probe Master Mix is an optimized and highly efficient reaction mixture developed for first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The master mix, formulated as a 2× reaction mixture, contains all components necessary for both cDNA synthesis and real-time PCR (including enzymes, dNTPs, stabilizers and enhancers), except primers, probes and RNA template. This master mix enables fast and highly reproducible procedures on the most common real-time PCR apparatus, from either total RNA or mRNA, and it was specifically developed for probe-detection technology. The latest developments in PCR enhancers have been incorporated in the NZYSpeedy One-step RT-qPCR Master Mix, including buffer chemistry and incorporation of highly robust engineered enzymes. This master mix does not contain ROX. For qPCR instruments that require this reference dye, please add ROX (Cat. No. MB406) according to the table shown in this brochure (ROX not provided).

Storage temperature

NZYSpeedy One-step RT-qPCR Probe Master Mix is shipped on dry ice and should be stored at -20 °C in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing in working aliquots.

Compatible real-time PCR instruments

The master mix 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™; iCycler®; iQ™5; Opticon™; Opticon™ 2

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

Roche:
Lightcycler® 96; Lightcycler® 480 & Lightcycler® Nano

Applied Biosystems (with addition of ROX):
7000; 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOne™plus; 7500; 7500 FAST; QuantStudio™ 6, 7, 12k Flex & ViiA7™

Protocol

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

NZYSpeedy One-step RT-qPCR Probe Master Mix (2x) (*)	10 µL	1×
10 µM forward primer	0.8 µL	400 nM
10 µM reverse primer	0.8 µL	400 nM
10 µM probe	0.2 µL	100 nM
Template	up to 8.2 µL	-
Nuclease-free water	up to 20 µL	-

(*) The master mix does not contain ROX, but addition of this internal passive reference dye can be conducted in a separate step. The final concentration will vary according to the qPCR instrument used. Please follow instructions described in the section below.

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.

ROX reference dye: NZYSpeedy One-step RT-qPCR Probe Master Mix (2x) is compatible with the majority of thermocyclers available in the market and can include ROX passive reference dye to normalize non-PCR-related fluctuations in fluorescence. If ROX addition is required for your qPCR platform, an optimal quantity of this dye should be included in your master mix. ROX can be purchased separately by NZYTech – Cat. No. MB406, in a ready-to-use solution of 25 µM. The recommended amount of ROX for the most common qPCR instruments is stated in the table below:

qPCR instrument	Volume of ROX per 1 mL of Master Mix (2x) (*)
Applied Biosystems: 7000/7300/7700/7900/7900HT/7900HT FAST/ StepOne™/StepOne™plus	28.5 µL
Applied Biosystems: 7500/7500FAST/QuantStudio™ 6, 7, 12k Flex/ViiA7™	3.8 µL
Bio-Rad®: CFX96™/CFX384™/iCycler®/iQ™5/Opticon™/ Opticon™ 2 **Qiagen:** Rotor-Gene™ 3000/6000/Q **Roche:** Lightcycler® 96/480/Nano	Not required

(*) For different volumes please scale-up or scale-down the volume of ROX accordingly.

Suggested thermal cycling conditions

NZYSpeedy One-step RT-qPCR Probe Master Mix 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	20 min	Reverse Transcription
1	95 °C	2-5 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s-50 s	Annealing/Extension

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 (RNase Cleaner, Cat. No. MB16001, can help removing RNases from surfaces and materials). 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 and probe: These guidelines refer to the design and set-up of dual labelled probes. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time RT-PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your real-time RT-qPCR experiment:

- Primers should have a melting temperature (T_m) of approximately 60 °C. The probe T_m should be approximately 10°C higher than that of the primers;
- The fragment to amplify should be between 80-200 bp in length and not superior to 300 bp;
- Final primer concentrations of 400 nM are suitable for most probe-based reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. Forward and reverse primers concentration should be equimolar;
- A final probe concentration of 100 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration;
- For multiplex RT-qPCR, probe concentrations in excess of 100 nM can result in cross channel fluorescence.

Template: It is important that the RNA template is purified and devoid of contamination by 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 (Cat. No. MB134).

$MgCl_2$: It is not necessary to supplement the reaction mixture with $MgCl_2$ as the NZYSpeedy One-step RT-qPCR Probe Master Mix already contains an optimized concentration of $MgCl_2$.

RT-qPCR optimization: it may be necessary to improve the efficiency of some reactions, such as multiplexing with more than two probes, or if the target amplicon is longer than 200 bp. In these cases, the reverse transcription reaction time can be extended up to 30 minutes; the annealing/extension time can be extended up to 60 seconds.

Quality control assays

Genomic DNA contamination

NZYSpeedy One-step RT-qPCR Probe Master Mix must be free of any detectable genomic DNA contamination as evaluated through a real-time PCR assay.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the master mix for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the master mix for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

NZYSpeedy One-step RT-qPCR Probe Master Mix is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

V2001

Certificate of Analysis

Test	Result
Genomic DNA contamination	Pass
Nuclease assays	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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