MB344_IFU_EN_V2402

One-step NZYSpeedy RT-qPCR Green kit, ROX plus

Catalogue number	Presentation
MB34401	1 mL (100 rxns of 20 µL)
MB34402	5 x 1 mL (500 rxns of 20 µL)

Description

One-step NZYSpeedy RT-qPCR Green kit, ROX plus 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, ROX plus developed for fast PCR, provided as a 2× reaction mixture, that contains all components necessary for real-time PCR, including a green intercalating dye, dNTPs, stabilizers and enhancers. In addition, a separate NZYRT mix which 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, ROX plus, 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 & Storage Conditions

The product is shipped in dry ice. Upon arrival, all components should be stored at -85 °C to -15 °C in a constant temperature freezer to guarantee maximal shelf life. Minimize the number of freeze-thaw cycles by storing both master mix and NZYRT mix in working aliquots. Do not expose the master mix to direct sunlight. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	MB34401		MB34402	
	TUBES	VOLUME	TUBES	VOLUME
One-step NZYSpeedy qPCR Green master mix (2x), ROX plus	1	1000 µL	5	1000 µL
NZYRT Mix	1	85 µL	5	85 µL
DEPC-treated H_2O	1	1000 µL	2	1000 µL

Specifications

Compatibility with real-time PCR instruments

One-step NZYSpeedy RT-qPCR Green kit, ROX plus is compatible with instruments that measure the passive reference signal. However, it is also compatible with instruments that do not require a passive reference signal for data normalization. It was optimized to be compatible with the following real-time PCR instruments:

Applied Biosystems: 7000; 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOne™plus

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: The inherent chemical instability of RNA and the ubiquitous presence of RNases require that particular care should be taken while working with RNA. We recommend using RNase-free plasticware/reagents and working in an RNase-free area (Nucleases & Nucleic Acid Cleaner, Cat. No. MB48301, or DNA & RNA Cleaner, Cat. No. MB46201, can help remove RNases from surfaces and materials).
- Handling instructions: 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. Any tubes containing amplified PCR product mustn't be opened in the PCR set-up area. Use sterile filtered tips. Minimize exposure by keeping reaction and components capped whenever possible.
- Controls: For verification of the absence of contamination, prepare a mixture sample without a DNA template (negative control). Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the qPCR reaction and detection system. The positive control should exhibit the expected amplification and/or fluorescence signal, confirming the assay's ability to accurately detect the target sequence. To avoid incorrect results caused by unwanted fluorescent substances, consider adding a No-Amplification Control tube. This tube should have the sample but not the enzyme master mix. Elevated fluorescence in the No-Amplification Control compared to the No-Template Control suggests potential fluorescent contaminants in either the sample or the thermal cycler's heat block. Moreover, we suggest that you always include no-RT control reactions, by replacing the NZYRT mix with DEPC-treated Water.

Procedure

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.

1. Thaw all components at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting.
2. In a clean reaction setup area, prepare the RT-qPCR reaction mixture (without template) according to the table below (please notice that the given volumes are based on a standard 20 µL final reaction mix and can be scale adjusted):
   Note 1: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed. Include sufficient reactions for the negative and positive controls.*
   Note 2: *If necessary, prepare a No-Amplification Control by adding DEPC-treated Water instead of the RT-qPCR master mix and/or a No-RT control reaction, by replacing the NZYRT mix with DEPC-treated Water.*
   Note 3: *We recommend performing replicates of all reactions.*

	1 REACTION VOLUME	**FINAL CONCENTRATION**
One-step NZYSpeedy qPCR Green master mix (2x), ROX plus	10 µL	1×
10 µM forward primer (not provided)	0.8 µL	400 nM (1)
10 µM reverse primer (not provided)	0.8 µL	400 nM (1)
NZYRT mix	0.8 µL	-
FINAL VOLUME =	up to 12.4 µL (2)	-

(1) Refer to the section of "Technical Notes" below for more details about primers' final concentrations in the reaction.
(2) If using smaller volumes for the primers, supplement the volume up to 12.4 µL with DEPC-treated Water.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Pipette 12.4 µL of the Mix into each well, according to your experimental plate/strip/tube configuration.
5. Pipette template (samples and controls):
   a. Add up to 7.6 µL of DNA sample/positive control into each respective well. If using less volume than 7.6 µL, supplement with DEPC-treated Water up to 7.6 µL. In total, the final volume of the reaction will be 20 µL.
   b. Add up to 7.6 µL of DEPC-treated Water for the negative control to achieve a final volume of 20 µL.

Note: *To avoid cross-contamination, we strongly recommend pipetting the template at last, preferably in a work separate area.*

6. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the real-time PCR detection steps.
7. Centrifuge briefly to spin down the contents and eliminate any air bubbles from the reaction mixtures.
8. Place the reaction plate/strip/tube within the real-time PCR instrument and run the general protocol defined below. These conditions might be adapted to suit your specific needs, within sensible limits.

Suggested thermal cycling conditions

One-step NZYSpeedy qPCR Green master mix (2x), ROX plus was optimized for the amplification of DNA fragments up to 200 bp under different RT-PCR cycling conditions. The table below displays a standard 1-step RT-qPCR setup optimized on several platforms. However, these conditions may be adapted to suit different equipment-specific protocols.

CYCLES	**TEMP.**	**TIME**	**STAGE**
1	50 °C	10 – 20 min (*)	Reverse transcription
1	95 °C	2 - 5 min	Polymerase activation
40	95 °C 60 °C	5 sec 30 sec (**)	Denaturation 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 by up to 20 minutes may be beneficial.
(**) Amplicons exceeding 200 base pairs in length can be employed, although optimizing extension times may be necessary. An additional increment in the extension step, up to 60 seconds, may also be required for complex assays.

Testing and Ct values

When comparing the One-step NZYSpeedy qPCR Green master mix (2x), ROX plus 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.

Technical Notes

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

- Primers should have a melting temperature (Tm) of approximately 58-62 °C;
- The fragment to amplify should be between 80-200 bp in length and not superior to 400 bp;
- Final primer concentrations of 400 nM are suitable for *green* reactions. However, to determine the optimal concentration we recommend titrating in the range 150-600 nM. Forward and reverse primers concentration should be equimolar;
- When possible design intron spanning primers to avoid gDNA amplification.

Template: The RNA template must be purified and devoid of contamination by RT-qPCR inhibitors (*e.g.* EDTA). The recommended amount of template is dependent upon the source 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 5 µg per 20 µL reaction.
- **mRNA:** purified mRNA can be used from 0.01 pg per 20 µL reaction.

To obtain a high yield of highly purified RNA we suggest using the NZY Viral RNA Isolation kit (Cat. No. MB407) or NZY Total RNA Isolation Kit (Cat. No. 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), ROX plus kit already contains an optimized concentration of $MgCl_2$.

RT-qPCR optimization: It may be necessary to improve the efficiency of some reactions. 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 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 PCR.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with all components 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

The One-step NZYSpeedy qPCR Green master mix (2x), ROX plus kit is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.