

PRODUCT INFORMATION

Thermo Scientific Verso SYBR Green 1-Step qRT-PCR Kit Plus ROX Vial

#AB-4104/A 200 x 25 µL

Lot _ Expiry Date _

Ordering Information

| Component | #AB-4104/A 200 rxns of 25 µL | #AB-4104/C 400 rxns of 25 µL |
|-------------------------|---------------------------------|---------------------------------|
| Verso Enzyme Mix | 50 µL | 100 µL |
| RT Enhancer | 250 µL | 500 µL |
| 2X 1-Step qPCR SYBR Mix | 2 × 1.25 mL | 5 mL |
| 1 mM ROX Reference Dye | 25 µL | 25 µL |
| 1 M MgCl ₂ | 100 µL | 100 µL |

Store at -20°C



Description

Thermo Scientific Verso SYBR® Green 1-Step qRT-PCR Kit Plus ROX Vial has been developed to quantify RNA in a single step assay. With the exception of primers and template, this kit contains in three vials all the components required to perform rapid, sensitive and reproducible qRT-PCR.

Verso™ Enzyme Mix

The Verso Reverse Transcriptase is active at high temperatures, is highly sensitive and can generate long cDNA strands. This mix also contains RNase inhibitor to protect RNA templates from degradation.

RT Enhancer is included to significantly improve the reverse transcription.

1-Step qPCR SYBR Mix, which contains:

- A proprietary reaction buffer which provides highly sensitive, specific and consistent fluorescence readings for real-time and end-point analysis. This buffer has been optimized to allow both reverse transcription and PCR amplification to occur in the same reaction across a wide range of templates.
- Thermo Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific ThermoPrime Plus DNA Polymerase, which prevents non-specific amplification during cDNA synthesis. Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). Thermo-Start requires an **activation step at 95°C for 15 minutes**.
- An inert blue dye to assist in the visualization of the 1-Step qPCR SYBR Mix after aliquoting into the reaction well.
- dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- SYBR Green I, a dye which fluoresces after binding to the double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.

ROX passive reference dye for normalization of data.



Verso Reverse Transcriptase

Verso is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity compared to *Reverse-iT™*. Verso synthesizes cDNA at a temperature range of 42°C to 57°C and is inactivated during the activation step of the Thermo-Start DNA Polymerase. Verso can reverse transcribe total RNA from 1 pg – 1 µg. The recommended amount of total RNA template to use in 1-step kits is between 1 pg - 100 ng.

ROX Dye

ROX is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR. A separate vial of ROX is included in this kit for optional addition to the 1-Step qPCR SYBR Mix. The final concentration will vary depending on the real time cycler. For example, for a concentration of 100 nM ROX in a final 1X qPCR reaction mix, dilute ROX (1 mM) 40 times i.e. 5 µL ROX Reference Dye + 195 µL PCR grade water and add 10 µL of the diluted ROX solution to each 1.25 mL vial of 1-Step qPCR SYBR Mix or 40 µL to each 5 mL vial of 1-Step qPCR SYBR Mix.

RT Enhancer

RT Enhancer greatly improves the efficiency of Verso as it stabilizes the enzyme on the template improving sensitivity.

Cycler Compatibility

Verso SYBR Green 1-Step qRT-PCR Kit Plus ROX Vial is compatible with all block-based qPCR instruments and the Rotor-Gene™.

MgCl₂

The initial concentration of MgCl₂ in the 1-Step qPCR SYBR Mix corresponds to 3 mM in the final 1X reaction. This concentration is effective over a broad range of templates. Some assays may be improved further with MgCl₂ optimization. A separate vial of 1 M MgCl₂ is therefore supplied with each kit. MgCl₂ concentration can be increased as follows: each 2.5 µL or 10 µL addition of MgCl₂ to the 1.25 mL or 5 mL undiluted 1-Step qPCR SYBR Mix respectively corresponds to an increase of 1 mM in the final 1X reaction. Scale up or down accordingly. Mix thoroughly by inverting the vial ten to twenty times. **Do not vortex.**

Storage Conditions

Store at -20°C until ready for use. Verso SYBR Green 1-Step qRT-PCR Kit Plus ROX Vial is stable for a minimum of 12 months. Avoid repeated freeze thawing. The ROX and SYBR® Green dyes are light sensitive; exposure should be minimized

Additional Info

The use of disposable gloves, RNase and DNase free filter tips and plastics is recommended.

For optimal results, the recommended amplicon length is in the range of 60 to 300 bp.

As best performance is achieved with dTTP, the 1-Step qPCR SYBR Mix contains a nucleotide mix with dTTP instead of dUTP.

DNase I treatment is recommended to remove double-stranded DNA.

Tips before use

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. **Do not vortex the 1-Step qPCR SYBR Mix or the Verso Enzyme Mix.** Briefly centrifuge to avoid bubbles within the wells, as these will interfere with the fluorescence. Always include a no template control (NTC) and a no enzyme control (NEC).

Protocol

Example of reaction mix preparation.

The volume of each component is for a 25 µL final reaction.

| | Volume | Final Concentration |
|-------------------------------|----------|---------------------|
| Verso Enzyme Mix | 0.25 µL | |
| 2X 1-Step qPCR SYBR Mix | 12.5 µL | 1X |
| RT Enhancer | 1.25 µL | |
| Forward primer (1 µM)* | 1.75 µL | 70 nM |
| Reverse primer (1 µM)* | 1.75 µL | 70 nM |
| Water, nuclease-free (#R0581) | To 25 µL | |
| Template (RNA)** | 1 - 5 µL | 1 ng |
| Total volume | 25 µL | |

*For optimization, a primer titration should be performed from 50 nM to 300 nM final concentration. Scale up or down the volume and concentration as appropriate.

**The amount of total RNA added as a template should be between 1 pg and 100 ng

Example of a 1-Step qRT-PCR thermal cycling program:

| | Temp. | Time | Number of cycles |
|----------------------------------|----------|--------|------------------|
| cDNA synthesis* | 50°C | 15 min | 1 cycle |
| Thermo-Start inactivation | 95°C | 15 min | 1 cycle |
| Denaturation | 95°C | 15 s | 40 cycle |
| Annealing** | 50-60 °C | 30 s | |
| Extension*** | 72 °C | 30 s | |

*Depending on the length of template and degree of secondary structure, the efficiency of the first strand synthesis maybe improved by optimizing temperature and time (42-57°C for 5-30 minutes).

**Annealing temperature depends on primer sequence.

***Time of extension depends on the length of the amplicon. If the amplicon exceeds 300 bp, amplification time should be adapted (Thermo-Start DNA Polymerase extends at approximately 1000 bp/min).

It is recommended to perform a melt curve to confirm the specificity of the reaction. Example of a melt curve program*:

| | | | |
|-----------------------|------|------|-----------|
| Denaturation | 95°C | 30 s | 1 cycle |
| Starting temp. | 60°C | 30 s | 1 cycle |
| Melting step** | 60°C | 10 s | 80 cycles |

*Melt curve program may vary depending on instrument manufacturer and software.

**Increase set point temperature by 0.5°C per cycle.

CERTIFICATE OF ANALYSIS

Verso Enzyme Mix and 1-Step qPCR SYBR Mix are tested functionally for use in qRT-PCR. The product must demonstrate linearity of amplification over a specified serial dilution of human total RNA.

Quality authorized by:  Jurgita Zilinskiene

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