Thermo SCIENTIFIC

PRODUCT INFORMATION

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

#AB-4100/A 200 x 25 µL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-4100/A 200 rxns of 25 µL	#AB-4100/C 400 rxns of 25 µL
Verso Enzyme Mix	50 µL	100 µL
RT Enhancer	250 µL	500 µL
2X 1-Step qPCR Mix	2 × 1.25 mL	5 mL
1 mM ROX Reference Dye	25 µL	25 µL

Store at -20°C

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Description

Thermo Scientific Verso 1-Step qRT-PCR Kit Plus ROX Vial has been developed to quantify RNA in a single step assay. With the exception of primers, template and probes, 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 remove contaminating DNA, eliminating the need for DNAse I treatment.

1-Step qPCR 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 Mix after aliquoting into the reaction well.
- dTTP to improve reaction sensitivity and efficiency compared to dUTP.

ROX passive reference dye for normalization of data.

Cycler Compatibility

Verso 1-Step qRT-PCR Kit Plus ROX Vial is compatible for use with any probe system and with all block-based qPCR instruments and the Rotor-Gene™.

Verso Reverse Transcriptase

Verso is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity. 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 Mix. The final concentration will vary dependent on each real time cycler manufacturers specification. For example, for a concentration of 100 nM ROX in a final 1X qPCR reaction mix, dilute 1 mM ROX 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 Mix or 40 µL to each 5 mL vial of 1-Step qPCR Mix.

RT Enhancer

RT Enhancer is included to remove contaminating DNA, eliminating the need for DNAse I treatment. It degrades double stranded DNA during the transcription of RNA and is inactivated during the activation step of the Thermo-Start DNA Polymerase.

Storage Conditions

Store at -20°C until ready for use. Verso 1-Step qRT-PCR Kit Plus ROX Vial is stable for a minimum of 12 months. Avoid repeated freeze thawing. The ROX dye is 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 Mix contains a nucleotide mix with dTTP instead of dUTP.
RT Enhancer is not required if DNase I treatment is performed prior to qRT-PCR.

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 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 Mix	12.5 µL	1X
RT Enhancer	1.25 µL	
Forward primer (10 µM)*	1 µL	400 nM
Reverse primer (10 µM)*	1 µL	400 nM
Probe	variable	100-250 nM
Template (RNA)**	1-5 µL	1 ng
Water, nuclease-free (#R0581)	To 25 µL	
Total volume	25 µL	

* For optimization, a primer titration should be performed from 100 nM to 500 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 100ng.

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 activation	95°C	15 min	1 cycle
Denaturation	95°C	15 s	40 cycles
Annealing/Extension**	60°C	60 s	

* Depending on the length of template and degree of secondary structure, the efficiency of the first strand synthesis may be improved by optimizing temperature and time (42-57°C for 5-30 minutes).
** Separate annealing (50–60°C for 30 sec) and extension steps (72°C for 30 sec) may be necessary with some probe systems (e.g. Molecular Beacons), as the optimal temperature for detecting fluorescence may be different.

CERTIFICATE OF ANALYSIS

Verso Enzyme Mix and 1-Step qPCR 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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