Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Verso 1-Step qRT-PCR ROX Kit

#AB-4101/C 400 x 25 µL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-4101/A 200 rxns of 25 µL	#AB-4101/C 400 rxns of 25 µL
Verso Enzyme Mix	50 µL	100 µL
RT Enhancer	250 µL	500 µL
1-Step qPCR-ROX Mix (2X)	2 × 1.25 mL	5 mL

Store at -20°C

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Description

Thermo Scientific Verso 1-Step qRT-PCR ROX Kit 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

Verso Enzyme Mix contains Verso Reverse Transcriptase and RNase inhibitor to protect RNA templates from degradation. Verso Reverse Transcriptase is highly sensitive RNA-dependent DNA polymerase with a significantly attenuated RNase H activity. Verso synthesizes long cDNA strands at a temperature range of 42°C to 57°C and is inactivated during the activation step of the Thermo Scientific 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.

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.

1-Step qPCR-ROX Mix 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-Start DNA Polymerase, a chemically modified hot-start version of Thermoprime Plus DNA Polymerase, which prevents non-specific amplification during cDNA synthesis. Thermo-Start requires an **activation step at 95°C for 15 minutes**. Thermo-Start has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).
- An inert blue dye to assist in the visualization of the 1-Step qPCR ROX Mix after aliquoting into the reaction well.
- dNTPs, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- ROX passive reference dye for normalization of data. The concentration of ROX in the final 1X reaction is 500 nM.

Cycler & Probe Compatibility

Verso 1-Step qRT-PCR ROX Kit is compatible for use with any probe system and with qPCR cyclers requiring high ROX dye level, including ABI PRISM® 7000, 7300, 7700, 7900, 7900HT and StepOne™.

Storage Conditions

Store at -20°C until ready for use. 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 ROX 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 ROX 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.

	Volume	Final Concentration
Verso Enzyme Mix	0.25 µL	
1-Step qPCR ROX Mix (2X)	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		100-250 nM
Template (RNA)	1-5 µL	1 pg - 100 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.

Example of a reverse transcription cycling protocol:

	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 ROX Mix are tested functionally for use in qRT-PCR. The product must demonstrate linearity of amplification over a specified serial dilution of control GAPDH RNA.

Quality authorized by:  Jurgita Zilinskiene

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Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.