Thermo SCIENTIFIC

PRODUCT INFORMATION

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

#AB-4106/A 200 x 25 µL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-4106/A 200 rxns of 25 µL	#AB-4106/C 400 rxns of 25 µL
Verso Enzyme Mix	50 µL	100 µL
RT Enhancer	250 µL	500 µL
2X 1-Step qPCR SYBR Low ROX Mix	2 × 1.25 mL	5 mL
1 M $MgCl_2$	100 µL	100 µL

Store at -20°C

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Description

Thermo Scientific Verso SYBR Green 1-Step qRT-PCR Low ROX Kit 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 RT-qPCR.

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 Low ROX 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 *Taq* 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 Low ROX 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.

Cycler Compatibility

Verso SYBR Green 1-Step qRT-PCR Low ROX Kit is compatible with all qPCR cyclers that require a ROX dye concentration of 25 nM, including ABI PRISM® 7500 (including Fast-Block) and Stratagene Mx4000®, Mx3000P®, Mx3005P™.

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. The concentration of ROX in the final 1X reaction is 25 nM.

RT Enhancer

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

$MgCl_2$

The initial concentration of $MgCl_2$ in the 1-Step qPCR SYBR Low ROX 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 $MgCl_2$ optimization. A separate vial of 1 M $MgCl_2$ is therefore supplied with each kit. $MgCl_2$ concentration can be increased as follows: each 2.5 µL or 10 µL addition of $MgCl_2$ to the 1.25 mL or 5 mL undiluted 1-Step qPCR SYBR Low ROX 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 Low ROX Kit 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 Low ROX 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 Low 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.
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 Low ROX 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
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 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 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**	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 may be 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 Low ROX Mix are tested functionally for use in RT-qPCR. The product must demonstrate linearity of amplification over a specified serial dilution of human total RNA.

Quality authorized by:  Jurgita Zilinskiene

NOTICE TO PURCHASER:

- Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,127,155, 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only), 5,994,056, 6,171,785. Use of this product in a passive reference method is covered by the following U.S. Patent: 5,928,907 (claim numbers 12-24, 27-28) and corresponding patent claims outside the US. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.
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- Use of Verso SYBR Green 1-Step qRT-PCR Low ROX Kit is licensed from bioMerieux, is covered by US Patent 5,654,143, US RE39031 and equivalents, and is for Research Use Only.

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