



PRODUCT INFORMATION

Thermo Scientific Verso 1-Step RT-PCR ReddyMix Kit

#AB-1454/LD/A 40 x 50 µL

Lot _ Expiry Date _

Ordering Information

Component	#AB-1454/LD/A 40 rxns of 50 µL	#AB-1454/LD/B 200 rxns of 50 µL
Verso Enzyme Mix	40 µL	200 µL
2X 1-Step PCR ReddyMix	1 mL	5 × 1 mL
RT Enhancer	100 µL	500 µL

Store at -20°C

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Description

Thermo Scientific Verso 1-Step RT-PCR ReddyMix Kit supplies in only two vials, all the components required to perform a rapid, sensitive and reproducible RT-PCR for the detection and analysis of RNA.

Verso™ Enzyme Mix includes RNase inhibitor to protect RNA templates from degradation and Verso Reverse Transcriptase, which is highly sensitive and active at high temperatures. Verso is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity. Verso can synthesize long cDNA strands, up to 11 kb, at a temperature range of 42°C to 57°C. 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.

2X 1-Step PCR ReddyMix™

2X 1-Step PCR ReddyMix is a proprietary reaction buffer which has been optimized to allow both reverse transcription and PCR amplification to occur in the same reaction across a wide range of templates. It contains Thermo Scientific ThermoPrime DNA Polymerase stable at high temperature. ThermoPrime has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). ReddyMix includes a dye and precipitant to facilitate the visualization and gel loading.

Rev.9

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 after 2 minutes at 95°C.

Storage Conditions

Store at -20°C until ready for use. Avoid repeated freeze thawing.

Additional Info

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

If DNase I treatment has been performed, RT Enhancer is not required.

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 Verso Enzyme Mix or the 1-Step PCR ReddyMix.**

Briefly centrifuge to avoid bubbles within the wells. 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 50 µL final reaction.

	Volume	Final Concentration
Verso Enzyme Mix	1 µL	
2X 1-Step PCR ReddyMix*	25 µL	1X
RT Enhancer	2.5 µL	
Forward primer (10 µM)**	1 µL	200 nM
Reverse primer (10 µM)**	1 µL	200 nM
Template (RNA)***	1-5 µL	1 ng
Water, nuclease-free (#R0581)	To 50 µL	
Total volume	50 µL	

* The gel precipitant in 1-Step PCR ReddyMix causes a slight increase in the thermal mass of the reaction mix. In a small number of cases this may necessitate some minor re-optimization of the thermal cycler program. If this is the case we suggest increasing the temperature of the denaturation step by 1–2°C and decreasing the temperature of the annealing step by 1–2°C. Alternatively, increase the duration of each step by 5–10 seconds.

**For optimization, a primer titration should be performed from 50 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 100 ng

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

	Temp.	Time	Number of cycles
cDNA synthesis*	50°C	15 min	1 cycle
Verso inactivation	95°C	2 min	1 cycle
Denaturation	95°C	20 s	35-45 cycles
Annealing**	50-60°C	30 s	
Extension***	72°C	1 min	
Final extension	72°C	5 min	1 cycle

* 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 1 kb amplification time should be adapted (ThermoPrime *Taq* DNA Polymerase extends at approximately 1 kb/min).

CERTIFICATE OF ANALYSIS

Verso 1-Step RT-PCR ReddyMix Kit is tested functionally for use in RT-PCR.

Quality authorized by:

 Jurgita Zilinskiene

NOTICE TO PURCHASER

- Use of this product is covered by US Patent No. 6,127,155. 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, no right to perform any patented method 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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PRODUCT USE LIMITATION

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