

# Thermo Scientific Verso 1-Step RT-PCR Hot-Start Kit

## **Description**

Verso<sup>™</sup> 1-Step RT-PCR Hot-Start 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<sup>TM</sup> Enzyme Mix includes Verso<sup>TM</sup> Reverse Transcriptase, which 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.
- 1-Step PCR Hot-Start Master Mix (2X), 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.
  - Thermo-Start<sup>TM</sup> DNA Polymerase, a chemically modified hot-start version of Thermoprime Plus DNA Polymerase, which prevents non-specific amplification during cDNA synthesis. Thermo-Start<sup>TM</sup> requires an **activation step at 95°C for 15 minutes**.
- <u>RT Enhancer</u> is included to remove contaminating DNA, eliminating the need for DNase I treatment.

### **Kit Contents**

Vial	Pack Size (cap color)	
	A	В
Verso Enzyme Mix	40μl (black)	200µl (black)
1-Step PCR Hot-Start Master Mix (2X)	1ml (purple)	5 x 1ml (purple)
RT Enhancer	100µl (yellow)	500μl (yellow)

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### INFORMATION

## Verso<sup>TM</sup> Reverse Transcriptase

Verso<sup>TM</sup> is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity compared to Reverse-iTTM. VersoTM can synthesize long cDNA strands, up to 11 kb, at a temperature range of 42°C to 57°C. Verso<sup>TM</sup> 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.

## Thermo-Start<sup>TM</sup> DNA Polymerase

The enzyme requires an activation step at  $95^{\circ}$ C for 15 minutes. Thermo-Start<sup>TM</sup> has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

### **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<sup>TM</sup> DNA Polymerase.

## **Storage Conditions**

Store at -20°C until ready for use. Verso™ 1-Step RT-PCR Hot-Start Kit is stable for a minimum of 12 months. Avoid repeated freeze thawing. Shipped on ice within the UK and on dry ice for international and within the US.

#### **Additional Info**

- The use of disposable gloves, RNase and DNase free filter tips and plastics is recommended.
- 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 Verso Enzyme Mix or the 1-Step PCR Hot-Start Master Mix.

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.

Reaction Mix

	Volume	Final Concentration
Verso Enzyme Mix	1 μl	
1-Step PCR Hot-Start Master Mix	25 µl	1X
(2X)	·	
Forward primer (10 µM) <sup>a</sup>	1 μl	200 nM
Reverse primer (10 µM) <sup>a</sup>	1 μl	200 nM
RT Enhancer	2.5 µl	
Water (PCR grade) b	variable	
Template (RNA) <sup>c</sup>	1 - 5 μl	1 ng
Total volume	50 μl	

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

	Temp.	Time	Number of cycle
cDNA Synthesis d	50°C	15 min	1 cycle
Thermo-Start activation	95°C	15 min	1 cycle
Denaturation	95°C	20 sec	
Annealing <sup>e</sup>	50-60°C	30 sec	35 - 45 cycles
Extension <sup>f</sup>	72°C	1 min	
Final extension	72°C	5 min	1 cycle

### **Notes**

- a 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.
- b The volume of the total reaction should be completed up to 50  $\mu l$  with water.
- $c-\mbox{The}$  amount of total RNA added as a template should be between 1pg and 100 ng.
- d 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).
- e Annealing temperature depends on primer sequence.
- f Time of extension depends on the length of the amplicon. If the amplicon exceeds 1 kb amplification time should be adapted (Thermo-Start<sup>TM</sup> DNA Polymerase extends at approximately 1 kb/min).



## **Quality control**

 $Verso^{TM} \ 1-Step \ RT-PCR \ Hot-Start \ Kit \ is \ tested \ functionally \ for \ use \ in \ RT-PCR.$ 

## **Ordering Information**

AB-1455/A	AB-1455/A Verso <sup>TM</sup> 1-Step RT-PCR Hot-Start Kit	
AB-1455/B	Verso™ 1-Step RT-PCR Hot-Start Kit	200 x 50 ul rxns

## **Related Products**

Cat. No.	Description	Quantity
AB-2400 AB-2400/W AB-2800 AB-2800/W AB-0745 AB-0626 AB-0558 AB-0386 AB-0783	ABgene® SuperPlate™ Semi-Skirted 96-Well PCR Plate ABgene® SuperPlate™ Semi-Skirted 96-Well PCR Plate, white ABgene® SuperPlate™ Skirted 96-Well PCR Plate ABgene® SuperPlate™ Skirted 96-Well PCR Plate, White Easy Peel (heat seal) Adhesive PCR Foil Adhesive PCR Film Strips of 8 Domed Caps Strips of 8 Flat Caps	25 plates 25 plates 25 plates 25 plates 100 sheets 100 sheets 100 strips 120 strips
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For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

Troubleshooting:	Email	Phone
North America (US, Canada, Central/South America)	Techservice.genomics @thermofisher.com	+1 (800) 235-9880
Europe (EU, Middle East, Africa)	Techservice.emea.genomics @thermofisher.com	(+) 44 1372 840410
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