Thermo Scientific Verso SYBR Green 1-Step QRT-PCR ROX Kit

Description

Verso™ SYBR® Green 1-Step QRT-PCR 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 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 significantly improve the reverse transcription.

1-Step QPCR SYBR 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-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**.
- An inert blue dye to assist in the visualization of the 1-Step QPCR SYBR 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.

INFORMATION

Kit Contents

Vial	Pack Size (cap color)		
	A	B	C
Verso Enzyme Mix	50µl (white)	500µl (white)	100µl (white)
RT Enhancer	250µl (yellow)	5 x 500µl (yellow)	500µl (yellow)
1-Step QPCR SYBR ROX Mix (2X)	2 x 1.25ml (green)	20 x 1.25ml (green)	5ml (clear)
$MgCl_2$ (1 M)	100µl (clear)	2 x 100µl (clear)	100µl (clear)

Verso™ Reverse Transcriptase

Verso™ is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity compared to *Reverse*-iT™. 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.

Thermo-Start™ DNA Polymerase

The enzyme 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).

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 500 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 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 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.**

Cycler Compatibility

Verso™ SYBR® Green 1-Step QRT-PCR ROX Kit is compatible with all QPCR cyclers that require a ROX dye concentration of 500 nM, including ABI PRISM® 7000, 7300, 7700, 7900 and 7900HT (including Fast-Block).

Storage Conditions

Store at -20°C until ready for use. Verso™ SYBR® Green 1-Step QRT-PCR 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. 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.
- 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 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 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
Reaction Mix	Verso Enzyme Mix	0.25 µl	
	1-Step QPCR SYBR ROX Mix (2X)	12.5 µl	1X
	RT Enhancer	1.25 µl	
	Forward primer (1 µM) [a]	1.75 µl	70 nM
	Reverse primer (1 µM) [a]	1.75 µl	70 nM
	Water (PCR grade) [b]	variable	
	Template (RNA) [c]	1 - 5 µl	1 ng
	Total volume	25 µl	

Example of a **1-Step QRT-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	15 sec	40 cycles
Annealing [e]	50-60°C	30 sec	
Extension [f]	72°C	30 sec	

It is recommended to perform a melt curve to confirm the specificity of the reaction.
Example of a **melt curve program** [g]:

Denaturation	95°C	30 sec	1 cycle
Starting temp.	60°C	30 sec	1 cycle
Melting step [h]	60°C	10 sec	80 cycles

Notes

a – 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.
b – The volume of the total reaction should be completed up to 25 µl with water.
c – The amount of total RNA added as a template should be between 1 pg 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 300 bp, amplification time should be adapted (Thermo-Start™ DNA Polymerase extends at approximately 1000 bp/min).
g – Melt curve program may vary depending on instrument manufacturer and software.
h – Increase set point temperature by 0.5°C per cycle.

Quality control

Verso™ Enzyme Mix and 1-Step QPCR SYBR ROX 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.

Ordering Information

AB-4105/A	Verso™ SYBR® Green 1-Step QRT-PCR ROX Kit	200 x 25 µl rxns
AB-4105/B	Verso™ SYBR® Green 1-Step QRT-PCR ROX Kit	2,000 x 25 µl rxns
AB-4105/C	Verso™ SYBR® Green 1-Step QRT-PCR ROX Kit	400 x 25 µl rxns

All formats are supplied with an additional vial of 1 M $MgCl_2$.

Related Products

Cat. No.	Description	Quantity
AB-1100/W	Thermo-Fast™ 96 PCR Detection Plate, white *	25 plates
AB-1400/W	Thermo-Fast™ 96 PCR Detection Plate Mark II, white *	25 plates
AB-1170	ABsolute™ QPCR Seal (adhesive seal)	50 sheets
AB-0812	Clear Seal Diamond (heat seal)	100 sheets
AB-0866	Ultra Clear Cap Strips (8 caps)	120 strips
AB-1154	Recombinant DNase I	2,000 units

* For Cycler compatibility and other color choices, see our latest catalogue or visit www.abgene.com

Troubleshooting

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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