

## Verso™ SYBR® Green 2-Step QRT-PCR Low ROX Kit

### Description

Verso™ SYBR® Green 2-Step QRT-PCR Low ROX Kit has been developed to quantify RNA in a 2-step QRT-PCR assay. With the exception of primers and template, this kit contains all the components required to generate high yields of cDNA from RNA and perform a sensitive QPCR.

- Verso™ Enzyme Mix includes Verso™ 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.
- 5X cDNA Synthesis Buffer, a proprietary reaction buffer which has been optimized to improve reverse transcription across a wide range of templates.
- Anchored oligo-dT primers and random hexamers provide flexible RNA priming methods for cDNA synthesis.
- RT Enhancer is included to remove contaminating DNA, eliminating the need for DNase I treatment.
- 2-Step QPCR SYBR Low ROX Mix a proprietary reaction buffer which provides highly sensitive, specific and consistent fluorescence readings for real-time and end-point analysis.

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 2-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 that fluoresces when bound to double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.

ROX, passive reference dye for normalization of data.

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INFORMATION

**Kit Contents**

| Vial                              | Pack Size (cap color) |                    |
|-----------------------------------|-----------------------|--------------------|
|                                   | A                     | B                  |
| Verso Enzyme Mix                  | 100µl (black)         | 2 x 100µl (black)  |
| 5X cDNA synthesis buffer          | 500µl (red)           | 2 x 500µl (red)    |
| Anchored oligo-dT (500 ng/µl)     | 100µl (orange)        | 2 x 100µl (orange) |
| Random hexamers (400 ng/µl)       | 100µl (blue)          | 2 x 100µl (blue)   |
| RT Enhancer                       | 100µl (yellow)        | 2 x 100µl (yellow) |
| 2-Step QPCR SYBR Low ROX Mix (2X) | 2 x 1.25ml (green)    | 5ml (clear)        |
| dNTP Mix (5 mM each)              | 200µl (purple)        | 2 x 200µl (purple) |
| MgCl <sub>2</sub> (1 M)           | 100µl (clear)         | 2 x 100µl (clear)  |

**Verso™ Reverse Transcriptase**

Verso™ is an RNA-dependent DNA polymerase with a significantly attenuated RNase H activity compared to *Reverse-iT™*. Verso™ can synthesize long cDNA strands, up to 11 kb, at a temperature range of 42°C to 57°C. The recommended amount of total RNA to use is between 1 µg and 1 µg.

**RNA Priming**

It is recommended that RNA primers be added to the final 1X reaction as follows: 1 µl of anchored oligo-dT (orange cap) or 1 µl of random hexamers (blue cap) or 1 µl of a blend of random hexamers and anchored oligo-dT 3:1 (v/v) or gene-specific primer (to final concentration of 0.5 – 2 µM).

Anchored oligo dT is not suitable for use with most prokaryotic RNA. In such cases, random hexamers or gene-specific primers are recommended.

**RT Enhancer**

RT Enhancer is included to remove contaminating DNA, eliminating the need for DNase I treatment. It degrades double stranded DNA during the cDNA synthesis step and is inactivated together with Verso™ at 95°C.

**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 25 nM.

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## MgCl<sub>2</sub>

The initial concentration of MgCl<sub>2</sub> in the 2-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<sub>2</sub> optimization. A separate vial of 1 M MgCl<sub>2</sub> is therefore supplied with each kit. MgCl<sub>2</sub> concentration can be increased as follows: each 2.5 µl or 10 µl addition of MgCl<sub>2</sub> to the 1.25 ml or 5 ml undiluted 2-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.**

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## Cycler Compatibility

Verso™ SYBR® Green 2-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™.

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## Storage Conditions

Store at -20°C until ready for use. Verso™ SYBR® Green 2-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. Shipped on ice within the UK and on dry ice for international and within the US.

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## 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 2-Step QPCR SYBR Low ROX Mix contains a nucleotide mix with dTTP instead of dUTP.
- RT Enhancer is not required if DNase I treatment is performed prior to cDNA synthesis.

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DIRECTIONS FOR USE

**Tips and Protocol**

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. **Do not vortex the 2-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).

**First Step**

The volume of each component is for a 20 µl final reaction.

|              |                                | Volume   | Final Concentration |
|--------------|--------------------------------|----------|---------------------|
| Reaction Mix | 5X cDNA synthesis buffer       | 4 µl     | 1X                  |
|              | dNTP Mix                       | 2 µl     | 500 µM each         |
|              | RNA Primer <sup>a</sup>        | 1 µl     |                     |
|              | RT Enhancer                    | 1 µl     |                     |
|              | Verso Enzyme Mix               | 1 µl     |                     |
|              | Water (PCR grade) <sup>b</sup> | variable |                     |
|              | Template (RNA) <sup>c, d</sup> | 1 - 5 µl | 1 ng                |
|              | Total volume                   | 20 µl    |                     |

Example of a reverse transcription cycling program:

|                             | Temp. | Time   | Number of cycle |
|-----------------------------|-------|--------|-----------------|
| cDNA Synthesis <sup>e</sup> | 42°C  | 30 min | 1 cycle         |
| Inactivation                | 95°C  | 2 min  | 1 cycle         |

Proceed to the second step (QPCR) or store cDNA samples at -20°C.

**Notes**

- a – For more information, please refer to the “RNA Priming” section on page 2.
- b – The volume of the total reaction should be completed up to 20 µl with water.
- c – To remove secondary structure, heat at 70°C for 5 minutes and place immediately on ice.
- d – The amount of RNA added as a template should be between 1 µg and 1 µg.
- e – 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 5-60 minutes).

## Second Step

The volume of each component is for a **25 µl final reaction**.

|              |                                    | Volume   | Final Concentration |
|--------------|------------------------------------|----------|---------------------|
| Reaction Mix | 2-Step QPCR SYBR Low ROX Mix (2X)  | 12.5 µl  | 1X                  |
|              | Forward primer (1 µM) <sup>a</sup> | 1.75 µl  | 70 nM               |
|              | Reverse primer (1 µM) <sup>a</sup> | 1.75 µl  | 70 nM               |
|              | Water (PCR grade) <sup>b</sup>     | variable |                     |
|              | Template (cDNA) <sup>c</sup>       | 1 µl     |                     |
| Total volume |                                    | 25 µl    |                     |

Example of a QPCR thermal cycling program:

|                         | Temp.   | Time          | Number of cycle |
|-------------------------|---------|---------------|-----------------|
| Thermo-Start activation | 95°C    | <b>15 min</b> | 1 cycle         |
| Denaturation            | 95°C    | 15 sec        | 40 cycles       |
| Annealing <sup>d</sup>  | 50-60°C | 30 sec        |                 |
| Extension <sup>e</sup>  | 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 <sup>f</sup>:

|                           |      |        |           |
|---------------------------|------|--------|-----------|
| Denaturation              | 95°C | 30 sec | 1 cycle   |
| Starting temp.            | 60°C | 30 sec | 1 cycle   |
| Melting step <sup>g</sup> | 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 – For standard templates only 1 µl should be added to reduce carryover of PCR inhibitors. This volume can be increased up to 5 µl for low copy number templates.
- d – Annealing temperature dependent on primer sequence.
- e – 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).
- f – Melt curve program may vary depending on instrument manufacturer and software.
- g – Increase set point temperature by 0.5°C per cycle.

## Quality control

Verso Enzyme Mix and 2-Step QPCR SYBR Low ROX Mix are tested functionally using QRT-PCR. The product must demonstrate linearity of amplification over a specified serial dilution of human total RNA.

## Ordering Information

| Cat. No.  | Description                                   | RT Reactions     | QPCR Reactions   |
|-----------|---|------------------|------------------|
| AB-4116/A | Verso™ SYBR® Green 2-Step QRT-PCR Low ROX Kit | 100 x 20 µl rxns | 200 x 25 µl rxns |
| AB-4116/B | Verso™ SYBR® Green 2-Step QRT-PCR Low ROX Kit | 200 x 20 µl rxns | 400 x 25 µl rxns |

All formats are supplied with an additional vial of 1 M MgCl<sub>2</sub>.

## Related Products

| Cat. No.  | Description  | Quantity   |
|-----------|--|------------|
| AB-0600/W | Thermo-Fast™ 96 Non-Skirted, white *                 | 25 plates  |
| 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 |

\* For Cycler compatibility and other color choices, see our latest catalogue or visit [www.abgene.com](http://www.abgene.com)

## Troubleshooting

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

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, 5,773,258, 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the US corresponding to US Patent No. 4,889,818. 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 (such as apparatus or system claims in US Patent No. 6,814,934) 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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