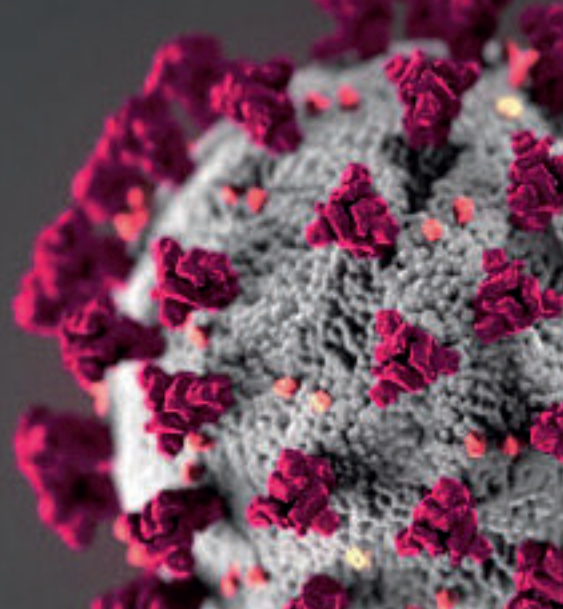


# RealQ Plus One-step RT-PCR Kit

*Optimized for sensitive detection*



RealQ Plus One-step RT-PCR is optimized for sensitive detection of viral RNA and low-copy RNAs.

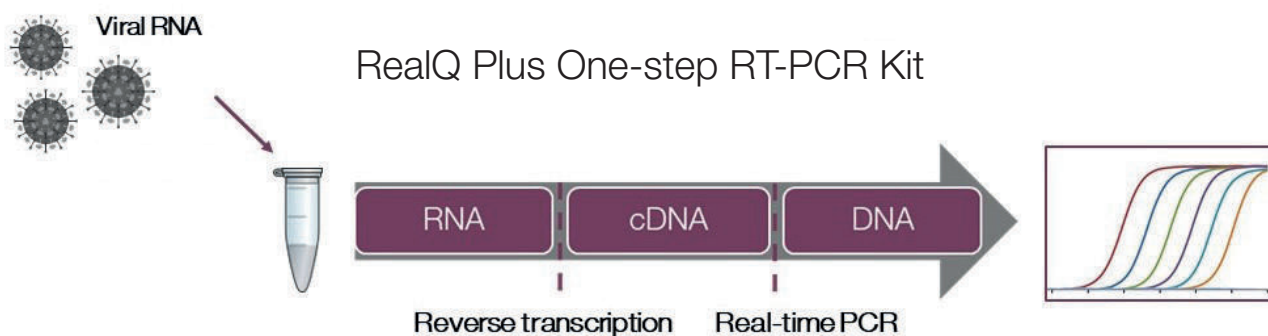
### Benefits:

- 1-step reverse transcription and real-time PCR set-up
- High sensitivity
- Efficient cDNA synthesis
- Low risk of contamination and pipetting errors
- Ideal for high-throughput testing
- Compatible with all standard real-time PCR Cyclers

The RealQ Plus One-step RT-PCR kit includes the RealQ Plus RT 5x PCR Mix, an optimized 20x RT mix and ROX internal reference dye to ensure user-flexibility and compatibility with all standard real-time PCR cyclers.

Efficient cDNA synthesis is ensured by the thermostable reverse transcriptase and the advanced RNase inhibitor blend.

High specificity is promoted by the TEMPase Hot Start DNA polymerase included in the RealQ Plus RT 5x PCR Mix.



### Applications:

- Detection of viral, low-copy RNA templates

| Product             | Size*          | Cat #   |
|---------------------|----------------|---------|
| RealQ Plus One-step | 200 Reactions  | A773302 |
| RT-PCR Kit          | 2000 Reactions | A773305 |
|                     | 4000 Reactions | A773306 |

\* Reaction size 20  $\mu$ l



## RealQ Plus One-step RT-PCR Kit



Cat. No.: A703302

A703302

200 Reactions (20 µl)

| Content     | 20x RT Mix | RealQ Plus RT 5x PCR Mix | ROX internal reference dye, 200 µM |
|-------------|------------|--------------------------|------------------------------------|
| ID          | 5700600    | 5030100                  | 5700300                            |
| Colour code | Yellow     | White                    | Amber                              |
| Size        | 1 x 0.4 ml | 1 x 0.8 ml               | 1 x 0.05 ml                        |

### Introduction

RealQ Plus One-step RT-PCR Kit includes RealQ Plus RT 5x PCR Mix, the 20x RT Mix, which is a blend of a thermostable reverse transcriptase and RNase inhibitors, and ROX internal reference dye to ensure user-flexibility and compatibility with all standard real-time PCR cyclers.

RealQ Plus RT 5x PCR Mix promotes high specificity and low background by using TEMPase Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

### Applications

- Detection of viral, low copy RNA templates

### Benefits

- 1-step reverse transcription and real-time PCR set-up
- High sensitivity
- Efficient cDNA Synthesis ensured by the thermostable Reverse Transcriptase and the advanced RNase inhibitor blend

### Pre-protocol Considerations

#### Protect RNA from degradation

- Take care to prevent RNA degradation by widely spread RNases.
- Prepare RNA samples in dedicated, but different area from the laboratory area used to set up reactions.
- Use nuclease free labware and gloves.

#### Check quality of RNA sample

Before cDNA synthesis, check RNA quality on a denaturing agarose gel to ensure good quality.

#### Amplicon size

Recommended amplicon size is less than 200 bp.

#### ROX reference dye

ROX serves as an internal reference for normalization of the fluorescent signal when using real-time PCR instruments, which can detect ROX.

**Table 1. Recommended final ROX concentrations vs. qPCR cyclers**

|   |
|---|
| <b>30 nM ROX:</b><br>Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx. |
| <b>300 nM ROX final concentration:</b><br>Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.                 |

\*For instruments not listed here, please contact technical support at [enzyme@ampliqon.com](mailto:enzyme@ampliqon.com)

If needed, prepare a fresh dilution of ROX internal reference dye. For a final reaction concentration of 30 nM dilute 200 µM ROX 1:100 in PCR grade water. For a final reaction concentration of 300 nM dilute 200 µM ROX 1:10 in PCR grade water. For a final reaction volume of 20 µl add 0.3 µl of the ROX dilution. The diluted ROX reference dye must be kept in a light-protected tube at 4 °C.

### Protocol

#### Note:

- Thaw and keep reagents on ice. Mix well before use.
- Keep your bench clean, wear gloves, use sterile tubes and filter pipette tips.

1. Prepare a 20 µl reaction by adding the components in the order shown in table 1.

**Table 1. Reaction components**

| Component                  | Vol./reaction   | Final concentration      |
|----------------------------|-----------------|--------------------------|
| Reverse primer             |                 | 100-400 nM final conc.   |
| Forward primer             |                 | 100-400 nM final conc.   |
| Specific Probe             |                 | 200 nM final conc.       |
| ROX 1:100 *                | 0.3 µl          | 30 nM – Low ROX          |
| ROX 1:10 *                 | 0.3 µl          | 300 nM – High ROX        |
| Total RNA or mRNA Template | X µl            | 1 pg – 1 µg or > 0.01 pg |
| RealQ Plus RT 5x MM        | 4 µl            | 1x                       |
| 20x RT Mix                 | 1-2 µl          | 1-2x**                   |
| PCR-grade H <sub>2</sub> O | Add up to 20 µl |                          |
| <b>TOTAL volume</b>        | 20 µl           |                          |

\*Optional – depending on applied real-time PCR instrument.

\*\*Increased amounts of RT-Mix (up to 2x) improve Cq values, but primer dimers might appear.

2. Gently mix without creating bubbles (do not vortex).
3. Place the reaction in the instrument and run the RT-PCR Program (See next page).

### RT-PCR Program

| Cycles | Duration of cycle     | Temperature     |
|--------|-----------------------|-----------------|
| 1      | Reverse transcription | 15 minutes      |
| 1      | Initial heating       | 50 – 55 °C      |
|        |                       | 95 °C           |
| 45     | Denaturation          | 5 – 15 seconds  |
|        | Annealing/Elongation  | 95 °C           |
|        |                       | 30 – 60 seconds |
|        |                       | 55 – 65 °C      |

### Recommended Storage

Long term storage at -20 °C.

## Related Products

| RealQ Plus One-step RT-PCR Kit (20 µl reactions) | Cat. No. |
|--|----------|
| 2000 Reactions                                   | A703305  |
| 4000 Reactions                                   | A703306  |

| Real-time PCR Master Mixes (400 x 25 µl reactions)  | Cat. No.                      |
|---|-------------------------------|
| RealQ Plus 2x Master Mix for probe, <ul style="list-style-type: none"><li>• without ROX™</li><li>• with low ROX™</li><li>• with high ROX™</li></ul> | A313402<br>A314402<br>A315402 |
| RealQ Plus 2x Master Mix Green <ul style="list-style-type: none"><li>• without ROX™</li><li>• with low ROX™</li><li>• with high ROX™</li></ul>      | A323402<br>A324402<br>A325402 |

| ROX and PCR Grade Water                       | Cat. No. |
|---|----------|
| ROX Internal Reference Dye 200 µM, 3 x 0.2 ml | A351513  |
| PCR Grade Water, 6 x 5 ml                     | A351513  |

Reagents for *in vitro* laboratory use only.

Other product sizes, combinations and customized solutions are available. Please look at [www.ampliqon.com](http://www.ampliqon.com) or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

**Made in Denmark**

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