

## Radiant™ Probe 1-Step Hi-ROX qPCR Kit

Catalog No.	Pack Size and Concentration	Components (Volume)	
		QH5001	100 x 20µl Reactions, 2X
QH5003	300 x 20µl Reactions, 2X	1-Step Hi-ROX 2X mix - 3 x 1mL	20X RTase – 3 x 0.2mL
QH5012	1200 x 20µl Reactions, 2X	1-Step Hi-ROX 2X mix - 12 x 1mL	20X RTase – 12 x 0.2mL

### Description

Alkali Scientific offers Radiant™ qPCR Kits, a high-performance portfolio of 2X ready-to-use real-time PCR reagents designed for best-in-class quantitative PCR. Radiant™ qPCR Kits are engineered for robust real-time PCR with earlier quantification cycle values (Ct), industry-leading sensitivity (increased limit of detection) and exceptional speed (rapid extension rates). The proprietary buffer system allows for highly efficient amplification of GC-rich and AT-rich sequences in addition to dramatic improvements in PCR sensitivity in low-copy assays and PCR conditions conducive to ultra-fast amplification. The Radiant™ Probe 1-Step Hi-ROX qPCR Kit is optimized for advanced multiplexing capability, delivering the same high efficiency, early Ct, and reaction speed even in complex multiplex assays. Furthermore, the 1-Step technology is based on a novel, thermostable reverse transcriptase and advances in PCR buffer technology (ionic strength, enhancers, and stabilizers).

- Novel hot-start chemistry for improved specificity and sensitivity.
- Next-generation PCR buffer formulations for maximum PCR efficiency and reaction speed.
- Broad range detection for increased reliability in low-copy assays

### Instrument Compatibility

The Radiant™ Probe 1-Step Hi-ROX qPCR Kit has been optimized for use with all probe chemistries including TaqMan™, FRET™, Scorpions and molecular beacon probes on qPCR instruments listed below. Each of these instruments has the capacity to analyze qPCR data with the passive reference signal either on or off.

Manufacturer	Model
Applied Biosystems®	Applied Biosystems® 7000, 7300, 7700, 7900HT, 7900HT Fast, StepOne™, StepOne™ Plus

### Components

The components of Radiant™ Probe 1-Step Hi-ROX qPCR Kit include 1-Step Hi-ROX 2X qPCR Mix (comprised of novel hot-start *Taq* DNA polymerase, proprietary real-time PCR buffer, optimized MgCl<sub>2</sub>, dNTPs, ROX reference dye, enhancers, and stabilizers) and 20X RTase (reverse transcriptase with blended RNase inhibitor).

### Storage

Radiant™ Probe 1-Step Hi-ROX qPCR Kit is shipped on blue or dry ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided. When stored as specified, Radiant™ Probe Hi-ROX qPCR Kit is stable for 12 months from date of receipt. The Kit may also be stored at 4°C for 1 month.

### Important Considerations

- Use primer-design software, such as Primer3 (<http://frodo.wi.mit.edu/primer3/>) or visual OMP™ (<http://dnasoftware.com/>). Primers should have a melting temperature (T<sub>m</sub>) of approximately 60°C and the T<sub>m</sub> of the probe should be approximately 10°C higher than that of the primers.
- Optimal amplicon length should be 80bp-200bp, and should not exceed 400bp.

## Reaction setup

1. Before starting, briefly vortex Radiant™ Probe 1-Step Hi-ROX 2X qPCR Mix.
2. Prepare a PCR master mix based on following table:

Component	20µl Reaction	Final Concentration/Notes
Radiant™ Probe 1-Step Hi-ROX 2X qPCR Mix	10 µl	1X
Forward Primer (10µM)	0.8 µl	400 nM
Reverse Primer (10µM)	0.8 µl	400 nM
Probe (10µM)	0.4 µl	200 nM
20X RTase	1.0 – 2.0µl	1X – 2X (1µl is recommended. 2.0µl may improve Ct and increase primer dimers)
Template RNA	1pg to 1µg total RNA >0.01pg mRNA	Variable

\* For alternative total reaction volumes (eg. 25 µl), scale all components proportionally and maintain final concentrations.

3. Program the instrument using the following conditions, acquiring data on the appropriate channel:

Cycles	Temperature & Time	Notes
1	45°C to 55°C, 10 minutes.	55°C should be used only for amplicons with complex secondary structure.
1	95°C, 2 minutes	Polymerase Activation
40	95°C, 5 seconds 60°C to 65°C, 20-30 seconds	Denaturation Anneal/Extension. Do not exceed 30 seconds and do not use temps below 60°C

## Quality Control

Radiant™ Probe 1-Step Hi-ROX qPCR Kit is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. Radiant™ Probe 1-Step Hi-ROX qPCR Kit is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

## Limitations of Use

This product is intended for research purposes only and is not intended for any animal or human therapeutic use.

## Technical Support

For Trouble-shooting and Technical Guidance, please contact us at [tech@alkalisci.com](mailto:tech@alkalisci.com) and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (amplification traces) if possible.

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