

## AzuraQuant™ Probe 1-Step qPCR Mix HiRox

Catalog No.	Pack Size and Concentration	Components and Volume	
<b>AZ-3901</b>	<b>100 x 20 µl reactions, 2x</b>	<b>2x 1-Step qPCR Mix HiRox - 1 x 1 mL</b>	<b>20x RTase 1 x 0.1 mL</b>
<b>AZ-3905</b>	<b>500 x 20 µl reactions, 2x</b>	<b>2x 1-Step qPCR Mix HiRox - 5 x 1 mL</b>	<b>20x RTase 5 x 0.1 mL</b>
<b>AZ-3910</b>	<b>1000 x 20 µl reactions, 2x</b>	<b>2x 1-Step qPCR Mix HiRox - 10 x 1 mL</b>	<b>20x RTase 10 x 0.1 mL</b>
<b>AZ-3950</b>	<b>5000 x 20 µl reactions, 2x</b>	<b>2x 1-Step qPCR Mix HiRox - 10 x 5 mL</b>	<b>20x RTase 10 x 0.5 mL</b>

### Description

The AzuraQuant™ Probe 1-Step qPCR Mix is a ready-to-use 2x master mix and companion thermostable reverse transcriptase for use in highly sensitive real-time RT-PCR assays and has been formulated for probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The AzuraQuant™ Probe 1-Step qPCR Mix is powered by Azura™ HS Taq DNA Polymerase, AzuraSprint™ Reverse Transcriptase, and an optimized buffer chemistry, providing robust first-strand cDNA synthesis and real-time PCR in a single tube. The Mix delivers early quantification cycle values (Ct) and broad range detection for increased sensitivity, speed, and reproducibility. The AzuraQuant™ Probe 1-Step qPCR Mix can be used to quantify a specific target RNA from either total RNA or mRNA while reducing the number of pipetting steps and time to result. The AzuraQuant™ Probe 1-Step qPCR Mix requires little if any optimization and employs a rapid antibody-mediated hot-start activation. In order to determine instrument compatibility and the most appropriate formulation, please refer to the qPCR Reagent Compatibility Tool.

### Storage

AzuraQuant™ Probe 1-Step qPCR Mix HiRox is shipped on blue ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided. When stored as specified, AzuraQuant™ Probe 1-Step qPCR Mix HiRox is stable for 12 months from date of receipt.

### Important Guidelines

- 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.
- Optimal amplicon length should be 80 bp-200 bp, and should not exceed 400 bp.
- Different real-time PCR instruments require different levels of ROX™ passive reference dye. Generally, modern instruments do not require passive reference but include the option to use it for normalization. Please refer to the qPCR Reagent Compatibility Tool to determine which kit is the most suitable for your instrument.
- When comparing AzuraQuant Probe 1-Step qPCR Mix with a reagent from an alternative supplier, we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of reaction speed.

## Reaction setup

1. Prepare a qPCR master mix based on following table. Briefly mix AzuraQuant™ Probe 1-Step qPCR Mix by inversion:

Component	20 µl Reaction	Final Concentration/Notes
AzuraQuant™ Probe 1-Step qPCR Mix HiRox	10 µl	1x
Forward Primer (10 µM)	0.8 µl	400 nM
Reverse Primer (10 µM)	0.8 µl	400 nM
Probe (10 µM)	0.4 µl	200 nM
20x RTase	1.0 µl	1x Add prior to RNA
Template RNA	1 pg to 1 µg Total RNA >0.01 pg mRNA	variable
PCR-grade water	Up to 20 µl final volume	

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

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

Cycles	Temperature & Time	Notes
1	50°C, 10 minutes	cDNA synthesis
1	95°C, 2 minutes	Enzyme activation
30 - 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

AzuraQuant™ Probe 1-Step qPCR Mix HiRox is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. AzuraQuant™ Probe 1-Step qPCR Mix HiRox is manufactured under a comprehensive quality management system, following ISO 9001:2015 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@azuragenomics.com](mailto:tech@azuragenomics.com) and provide qPCR reaction conditions, cycling parameters, amplicon size, and screen grabs (amplification traces and melting profiles) if possible.

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