AzuraQuant™ Probe One-Step qPCR Mix HiRox

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-3901	100 x 20 µl reactions, 2x	2x One-Step qPCR Mix HiRox - 1 x 1ml	20x RTase 1 x 0.1ml
AZ-3905	500 x 20 µl reactions, 2x	2x One-Step qPCR Mix HiRox - 5 x 1ml	20x RTase 5 x 0.1ml
AZ-3910	1000 x 20 µl reactions, 2x	2x One-Step qPCR Mix HiRox - 10 x 1ml	20x RTase 10 x 0.1ml

Description

The AzuraQuant™ Probe One-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 One-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 earlier quantification cycle values (Ct) and broad range detection for increased sensitivity, speed, reliability and reproducibility. The AzuraQuant™ Probe One-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 One-Step qPCR Mix requires little if any optimization and employs rapid antibody-mediated hot-start activation and a processive enzyme chemistry, providing unmatched performance in multiplexing. In order to determine instrument compatibility and the most appropriate ROX™ variant, please refer to AzuraQuant™ Selection Guide.

Storage

AzuraQuant™ Probe One-Step qPCR Mix HiRox 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, AzuraQuant™ Probe One-Step qPCR Mix HiRox is stable for 12 months from date of receipt. The 2x Mix may also be stored at 4°C for 1 month.

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 (Tm) of approximately 60°C. The Tm of the probe should be approximately 6°C - 10°C higher than that of the primers. For Taqman™ probes, choose a probe close to the 5' primer and avoid terminal guanosine residues.
- Optimal amplicon length should be 80bp-200bp, and should not exceed 400bp.
- 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 AzuraQuant Selection Guide to determine which kit is the most suitable for your instrument (http://www.azuragenomics.com/azuraquant-selection).
- When comparing AzuraQuant Probe One-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 (and briefly vortex AzuraQuant™ Probe One-Step qPCR Mix before use):

Component	20µl Reaction	Final Concentration/Notes
AzuraQuant™ Probe One-Step qPCR Mix HiRox	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 – 2.0 µl	1x or 2x 2.0 µl will improve Ct
Template RNA	1pg to 1µg Total RNA >0.01pg 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	45°C to 55°C, 10 minutes	55°C for amplicons with high degree of secondary structure
1	95°C, 2 minutes	Enzyme activation; use 3 minutes for genomic DNA
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 One-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 One-Step qPCR Mix HiRox 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@azuragenomics.com and provide qPCR reaction conditions, cycling parameters, amplicon size, and screen grabs (amplification traces and melting profiles) if possible.