AzuraQuant™ Probe Fast qPCR Mix NoRox

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-2901	100 x 20 µl reactions, 2x	AzuraQuant Probe Fast qPCR Mix NoRox - 1 x 1ml
AZ-2905	500 x 20 µl reactions, 2x	AzuraQuant Probe Fast qPCR Mix NoRox - 5 x 1ml
AZ-2920	2000 x 20 µl reactions, 2x	AzuraQuant Probe Fast qPCR Mix NoRox - 20 x 1ml

Description

The AzuraQuant™ Probe Fast qPCR Mix NoRox is a ready-to-use 2x master mix for use in real-time quantitative PCR assays and has been formulated for probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The AzuraQuant™ Probe Fast qPCR Mix NoRox contains Azura HS Taq DNA Polymerase and an optimized buffer chemistry providing robust real-time PCR with earlier quantification cycle values (Ct) and broad range detection for increased sensitivity, speed, reliability and reproducibility. In addition, the mix exhibits superior sensitivity in complex multiplex reactions, in which multiple amplicons can be detected without a loss in efficiency. The AzuraQuant™ Probe Fast qPCR Mix NoRox requires little if any optimization and can be used to quantify any DNA templates including cDNA, genomic DNA, and low copy viral sequences while providing exceptional resistance to many PCR inhibitors. The master mix also employs rapid antibody-mediated hot-start activation and processive enzyme chemistry, making it compatible with both standard and fast instrument cycling programs. In order to determine instrument compatibility and the most appropriate ROX™ variant, please refer to the AzuraQuant™ Selection Guide.

- Probe-based detection of DNA/cDNA, Gene Expression analysis, Multiplex qPCR and Two-Step RT-qPCR, and Detection of sequence variants
- Compatible with standard and fast cycling instruments and a wide range of cycling parameters.
- Hot-Start chemistry reduces primer-dimer formation and allows room-temperature assembly.
- Advanced formulation provides unrivalled sensitivity in singleplex as well as complex multiplex assays.

Storage

AzuraQuant™ Probe Fast qPCR Mix NoRox 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 Fast qPCR Mix NoRox 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 and the Tm of the probe should be approximately 6° - 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 Fast 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 Fast qPCR Mix NoRox before use):

Component	20µl Reaction	Final Concentration/Notes
AzuraQuant™ Probe Fast qPCR Mix NoRox	10 µl	1x
Forward Primer (10µM)	0.8 µl	400 nM
Reverse Primer (10µM)	0.8 µl	400 nM
Probe (10µM)	0.4 µl	200nM
Template DNA	<100ng cDNA, <1µg genomic DNA	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	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 Fast qPCR Mix NoRox is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. AzuraQuant™ Probe Fast qPCR Mix NoRox 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.