

## AzuraQuant™ Green Fast qPCR Mix HiRox

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-2001	100 x 20 µl reactions, 2x	2x AzuraQuant Green Fast qPCR HiRox - 1 x 1 mL
AZ-2005	500 x 20 µl reactions, 2x	2x AzuraQuant Green Fast qPCR HiRox - 5 x 1 mL
AZ-2020	2000 x 20 µl reactions, 2x	2x AzuraQuant Green Fast qPCR HiRox - 20 x 1 mL
AZ-2050	5000 x 20 µl reactions, 2x	2x AzuraQuant Green Fast qPCR HiRox - 10 x 5 mL

### Description

The AzuraQuant™ Green Fast qPCR Mix HiRox is an ultra-stable 2x master-mix comprised of a non-inhibitory intercalating dye, Azura HS Taq DNA Polymerase and a highly optimized reaction buffer for use in SYBR™ real-time quantitative PCR assays. The AzuraQuant™ Green Fast qPCR Mix HiRox reliably quantifies cDNA and genomic DNA and is formulated for room temperature stability and increased resistance to freeze-thaw cycles. The ready-to-use 2x master mix delivers an increased limit of detection with best-in-class sensitivity. In order to determine instrument compatibility and the most appropriate formulation, please refer to the qPCR Reagent Compatibility Tool.

- Fluorescent detection of DNA/cDNA, Gene Expression analysis, 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.
- Optimized buffer chemistry allows detection of low-copy targets with earlier quantification values.

### Storage

AzuraQuant™ Green Fast 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™ Green Fast qPCR Mix HiRox is stable for 18 months from date of receipt. The 2x Mix may also be stored at 4°C for 4 months or 23°C for up to 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 (T<sub>m</sub>) of approximately 60°C.
- 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 qPCR Reagent Compatibility Tool to determine which kit is the most suitable for your instrument.
- When comparing AzuraQuant Green 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™Green Fast qPCR Mix HiRox before use):

Component	20µl Reaction	Final Concentration/Notes
2x AzuraQuant™Green Fast qPCR HiRox	10 µl	1x
Forward Primer (10 µM)	0.8 µl	400 nM
Reverse Primer (10 µM)	0.8 µl	400 nM
Template DNA	<100 ng cDNA, <1 µg 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 SYBR® Green or FAM 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)
Melt Analysis (Optional)		

## Quality Control

AzuraQuant™Green Fast 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™Green Fast 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](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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