

AzuraQuant™ Genotyping Mix HiRox

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-3101	100 x 20 µl reactions, 2x	AzuraQuant Genotyping Mix HiRox - 1 x 1ml
AZ-3105	500 x 20 µl reactions, 2x	AzuraQuant Genotyping Mix HiRox - 5 x 1ml
AZ-3120	2000 x 20 µl reactions, 2x	AzuraQuant Genotyping Mix HiRox - 20 x 1ml

Description

The AzuraQuant™ Genotyping Mix is designed for best-in-class use in dual-labeled probe based genotyping assays and is fully compatible with Applied Biosystems TaqMan® Pre-Designed SNP Genotyping assays. The AzuraQuant™ Genotyping Mix utilizes the latest advancements in enzyme purification and buffer optimization to ensure that the AzuraQuant™ Genotyping Mix produces highly-specific, ultra-sensitive qPCR with clear allelic discrimination and outstanding allele clustering. The Mix has been developed and optimized for precise, fast, and highly reproducible genotyping of sequence variants including Type IV SNP (single nucleotide polymorphisms). The AzuraQuant™ Genotyping Mix requires little if any optimization and provides an ideal buffer environment for very tight binding of allele-specific probes. In order to determine instrument compatibility and the most appropriate ROX variant, please refer to [AzuraQuant™ Selection Guide](#).

- **Accurate Allelic Discrimination:** Tight clustering for high confidence genotype calling
- **Flexibility:** Fully compatible with TaqMan® dual-labeled probes
- **Optimized for Difficult Templates:** Efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

Storage

AzuraQuant™ Genotyping 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™ Genotyping 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 (T_m) of approximately 60°C and the T_m 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>)

Reaction setup

1. Prepare a qPCR master mix based on following table (and briefly vortex AzuraQuant™ Genotyping Mix HiRox before use):

Component	20µl Reaction	Final Concentration/Notes
AzuraQuant™ Genotyping 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	200nM
Template DNA	Up to 20pg 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, 3 minutes	Enzyme activation; use 3 minutes for genomic DNA
30 - 40	95°C, 15 seconds 55°C to 60°C, 60 seconds	Denaturation Anneal/Extension (do not exceed 60 seconds)

Quality Control

AzuraQuant™ Genotyping Mix HiRox is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. AzuraQuant™ Genotyping 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.

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