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Empowering Discovery

AzuraQuant™ HRM Fast Mix

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-3401	100 x 20 µl reactions, 2x	2x AzuraQuant HRM Fast Mix - 1 x 1ml
AZ-3405	500 x 20 µl reactions, 2x	2x AzuraQuant HRM Fast Mix - 5 x 1ml
AZ-3420	2000 x 20 µl reactions, 2x	2x AzuraQuant HRM Fast Mix - 20 x 1ml

Description

The AzuraQuant™HRM Fast Mix is a ready-to-use 2x master mix for use in High-throughput, Fast High-Resolution Melt (HRM) for the analysis of mutations, polymorphisms and epigenetic differences in double-stranded DNA. The system contains Azura™ HS Taq polymerase, high affinity antibodies and Vivid Green™ 2 dye, a novel saturating, third-generation dye which is ideal for accurate SNP genotyping. The AzuraQuant™HRM Fast Mix has been developed and optimized for fast PCR cycling, improved sensitivity, and maximum discrimination between sequence variants. The latest advances in enzyme preparation coupled with the optimization of ionic conditions and enhancers enable the amplification and clear discrimination of even the most challenging sequence variations including Class IV SNP (Single Nucleotide Polymorphisms) without sequence preference.

- **Sensitivity**: Accurately detect/discriminate Class IV (A/T) SNP mutations.
- **Highly Reproducible**: Highly specific and developed to maximize sequence differences
- **PCR Efficiency**: Specific and Robust Amplification from GC and AT rich sequences

Storage

AzuraQuant™ HRM Fast Mix 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™ HRM Fast Mix 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.
- Optimal amplicon length should be 80bp-200bp, and should not exceed 400bp.
- For users to perform HRM based genetic analysis, instruments which provide High Resolution Melt functionality include the following: MyGo Pro, MyGo Mini, BioRad® CFX96™, CFX384™, Roche Lightcycler® 480 and Nano, Applied Bosystems 7900HT, StepOne™, StepOne™ Plus, 7500, 7500 FAST, Viia7™, Eppendorf Mastercycler®, and Qiagen/Corbett 6000 and Q.

Reaction setup

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

Component	20µl Reaction	Final Concentration/Notes
2x AzuraQuant™ HRM Fast Mix	10 µl	1x
Forward Primer (10µM)	0.8 µl	400 nM
Reverse Primer (10µM)	0.8 µl	400 nM
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 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
HRM Analysis (Instrument Instructions and optional melt profile)		

Quality Control

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