AzuraQuant™ Green Fast qPCR Mix LoRox

Catalog No: AZQ-Lo
Lot No: XXXXX
Supplied as: liquid
Stability: store at -20°C

Background

The AzuraQuant™ Green Fast qPCR Mix LoRox is a ready-to-use 2x master mix for use in real-time quantitative PCR assays in which intercalating dye-based detection provides the option of a post amplification melt profile. The system contains Vivid Green™ dye, a novel fluorescent DNA-binding dye which produces minimal PCR inhibition and greater fluorescence upon binding to double stranded DNA than SYBR™ Green I. The AzuraQuant™ Green Fast qPCR Mix LoRox contains Azura HS Taq DNA-Polymerase, an optimized buffer chemistry, and a proprietary DNA-binding dye providing robust real-time PCR with earlier quantification cycle values (Ct) and broad range detection for increased sensitivity, speed, reliability and reproducibility. The AzuraQuant™ Green Fast qPCR Mix LoRox requires little if any optimization and can be used to quantify any DNA templates including cDNA, genomic DNA, and low copy viral targets.

- 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.

Important Guidelines

- Use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMPTM (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C.
- Optimal amplicon length should be 80 bp - 200 bp, and should not exceed 400 bp.
- 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.
- 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 LoRox before use):

Component	20 µL Reaction	Final Concentration/Notes
2x AzuraQuant™ Green Fast qPCR LoRox	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	Denaturation
	60°C to 65°C, 20 – 30 seconds	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 LoRox is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Usage

This product is offered by Biomol for research purposes only. Not for diagnostic purposes or human use. It may not be resold or used to manufacture commercial products without written approval of Biomol GmbH.