

AzuraView™ GreenFast qPCR Blue Mix LR

Catalog No.	Pack Size and Concentration	Components and Volume
AZ-2301	100 x 20 µl reactions, 2x	2x AzuraView GreenFast qPCR Blue Mix LR - 1 x 1ml
AZ-2305	500 x 20 µl reactions, 2x	2x AzuraView GreenFast qPCR Blue Mix LR - 5 x 1ml
AZ-2320	2000 x 20 µl reactions, 2x	2x AzuraView GreenFast qPCR Blue Mix LR - 20 x 1ml

Description

The AzuraView™ GreenFast qPCR Blue Mix LR is a ready-to-use, ultra-stable 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 AzuraView™ GreenFast qPCR Blue Mix LR contains Azura HS Taq DNA Polymerase, an optimized buffer chemistry with low ROX, 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, and room temperature stability. The AzuraView™ GreenFast qPCR Blue Mix which contains an inert blue tracer dye to facilitate pipetting can be used to quantify any DNA templates including cDNA, genomic DNA, and low copy viral targets. In order to determine instrument compatibility and the most appropriate ROX variant, please refer to the [AzuraView™ Selection Guide](#).

- Fluorescent detection of DNA/cDNA, Gene Expression analysis, and Detection of sequence variants
- Room Temperature Stable for 30 days
- Hot-Start chemistry reduces primer-dimer formation and allows room-temperature assembly.
- Contains an inert Blue Tracer Dye to facilitate pipetting and visualization in plates.

Storage

AzuraView™ GreenFast qPCR Blue Mix LR 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, AzuraView™ GreenFast qPCR Blue Mix LR 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 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.
- Ideally, the 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 AzuraView Selection Guide to determine which kit is the most suitable for your instrument.
- When comparing AzuraView™ GreenFast qPCR Blue Mix LR 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 AzuraView™ GreenFast qPCR Blue Mix LR before use):

Component	20µl Reaction	Final Concentration/Notes
2x AzuraView™ GreenFast qPCR Blue Mix LR	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 – 5 minutes	Enzyme activation; use > 3 minutes for genomic DNA
30 - 40	95°C, 5 seconds 60°C to 65°C, 10 - 40 seconds	Denaturation Anneal/Extension (do not exceed 40 seconds and do not use temps below 60°C for optimal results)
Melt Analysis (Optional)		

Quality Control

AzuraView™ GreenFast qPCR Blue Mix LR is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. AzuraView™ GreenFast qPCR Blue Mix LR 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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