Product Testing Report

BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890)

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Multiplex Amplification Performance

Product Description

BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890) is a probe-based quantitative PCR mastermix optimized for sensitive and reproducible real-time PCR detection in both singleplex and multiplex applications. The formulation contains a hot-start DNA polymerase, optimized reaction buffer, Mg^{2+}, dNTPs, and proprietary enhancers designed to support efficient amplification across multiple fluorescent detection channels.

This study evaluates the multiplex amplification performance of the mastermix using four independent hydrolysis probe assays labeled with spectrally distinct fluorophores.

Objective

To evaluate the performance of BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890) in both singleplex and multiplex qPCR applications using four probe-based assays targeting HPV genotypes and a human genomic DNA internal control.

The study was designed to assess:

- Singleplex amplification performance of each assay independently
- Simultaneous amplification of four targets within a multiplex reaction
- Fluorophore channel compatibility
- Multiplex amplification efficiency and signal discrimination
- Compatibility with viral DNA and human genomic DNA templates

Materials and Reagents

- BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890)
- HPV16 HEX probe and primer set
- HPV18 ROX probe and primer set
- HPV56 FAM probe and primer set
- Human β-Actin CY5 probe and primer set
- HPV16 DNA template
- HPV18 DNA template
- HPV56 DNA template
- Human genomic DNA template
- Nuclease-free water
- Real-time PCR instrument capable of FAM, HEX, ROX, and CY5 detection (BioRad® CFX96™ Real-Time PCR Detection Systems)

Target and Fluorophore Configuration

Target	Fluorophore
HPV16	HEX
HPV18	ROX
HPV56	FAM
Human β-Actin	CY5

Method Summary

Singleplex qPCR Evaluation

Each probe and primer set was first evaluated independently in singleplex qPCR reactions using BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890).

Reaction mixtures contained:

- 1X BlasTaq™ Probe 2X qPCR MasterMix
- Forward and reverse primers
- Corresponding hydrolysis probe
- Template DNA
- Nuclease-free water

Template inputs were:

- HPV16: 10^4 copies/reaction
- HPV18: 10^4 copies/reaction
- HPV56: 10^4 copies/reaction
- Human β-Actin: 10 ng/µL human genomic DNA

Amplification was performed using standard probe-based qPCR cycling conditions.

Multiplex qPCR Evaluation

A multiplex qPCR reaction containing all four primer/probe sets was assembled to evaluate simultaneous target amplification performance.

The multiplex reaction contained:

- HPV16 HEX assay
- HPV18 ROX assay
- HPV56 FAM assay
- Human β-Actin CY5 assay
- Corresponding DNA templates
- BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890)

All four targets were amplified simultaneously within a single reaction using standard multiplex qPCR cycling conditions.

The selected assays utilize four spectrally distinct fluorophores commonly employed in multiplex qPCR workflows. Initial singleplex validation was performed to confirm proper amplification and fluorescent signal generation for each individual assay.

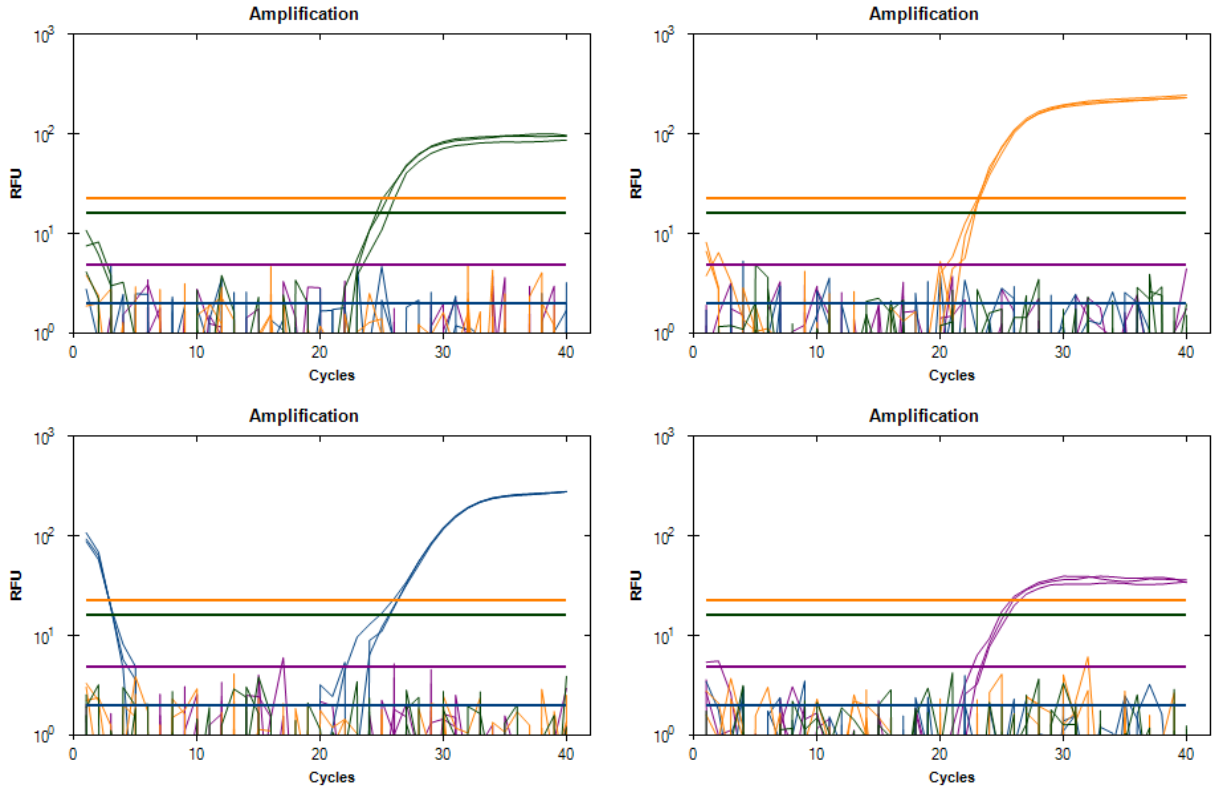
Subsequently, all assays were combined into a single multiplex reaction to evaluate the ability of the mastermix to support simultaneous amplification and detection across multiple fluorescent channels without significant signal interference.

The inclusion of a human β-Actin internal control additionally demonstrates compatibility with genomic DNA templates commonly used in multiplex assay normalization and sample quality assessment workflows.

Results Summary

Singleplex Reactions

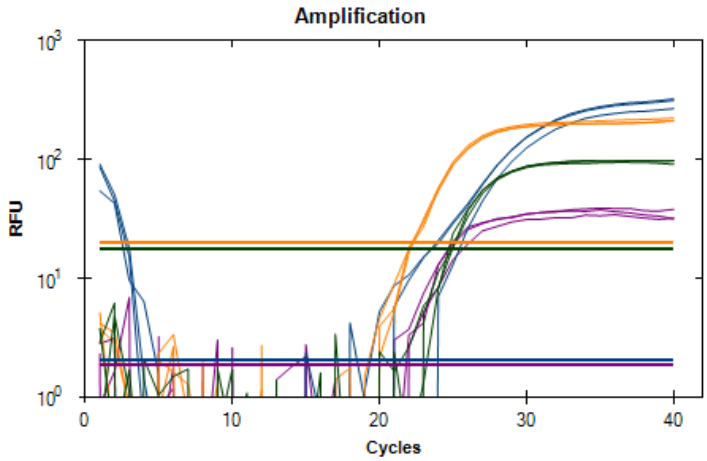
Figure 1. Singleplex qPCR Amplification of Individual Targets. Representative singleplex amplification curves for HPV16 (HEX, Green), HPV18 (ROX, Yellow), HPV56 (FAM, Blue), and human β-Actin (CY5, Purple) generated using BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890).



Target	Fluorophore	Target Input	Ct Value	Observed Result	Status
HPV16	**HEX**	10^4 copies	25.7	Successful amplification	Pass
HPV18	**ROX**	10^4 copies	22.9	Successful amplification	Pass
HPV56	**FAM**	10^4 copies	23.3	Successful amplification	Pass
Human β-Actin	**CY5**	10 ng gDNA	22.2	Successful amplification	Pass

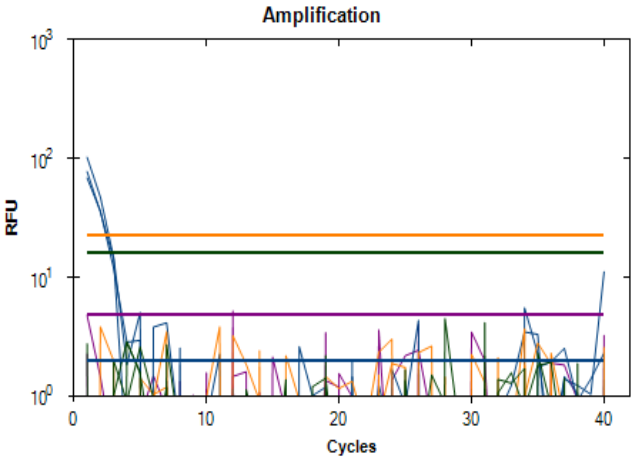
Multiplex Reactions

Figure 2. Representative multiplexplex qPCR amplification plot showing simultaneous detection of HPV16 (Hex, Green), HPV18 (Rox, Yellow), HPV56 (Fam,Blue), and human β-Actin (Cy5, Purple) targets in a single reaction using BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890).



Target	Fluorophore	Target Input	Ct Value	Observed Result	Status
HPV16 + HPV18 + HPV56 + β-Actin	HEX, ROX. FAM, CY5	10^4 copies each of the HPV and 10 ng Human gDNA	HEX – 24.8 ROX – 22.22 FAM – 20.99 CY5 – 21.31	Simultaneous amplification detected across all four fluorescent channels	Pass

Figure 3. NTC (No Template Control) (FAM, HEX, ROX, Cy5) shows no detectable amplification, confirming absence of contamination and non-specific amplification across all assay formats and channels.



Conclusion

All four assays demonstrated successful amplification in independent singleplex reactions using BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890). Distinct amplification signals were observed for HPV16 (HEX), HPV18 (ROX), HPV56 (FAM), and human β-Actin (CY5), confirming compatibility of the mastermix with multiple fluorescent detection channels.

When combined into a multiplex format, all four targets were simultaneously amplified and detected within a single reaction. The observed amplification profiles demonstrate that the mastermix supports multiplex hydrolysis probe-based qPCR workflows involving concurrent detection across FAM, HEX, ROX, and CY5 channels without significant signal suppression or channel interference under the tested conditions.

Successful amplification of both viral DNA targets and human genomic DNA further supports compatibility with multiplex molecular detection assays requiring internal control monitoring.

Under the tested conditions, BlasTaq™ Probe 2X qPCR MasterMix (Cat. G890) demonstrated robust performance in both singleplex and multiplex probe-based qPCR applications and met internal QC criteria for multi-target fluorescent detection workflows.