Take Multiplex QPCR to the Next Level

Data Sheet

- Simultaneously amplify low & high abundance targets
- Maximize analysis of limited or rare samples
- Save time and increase throughput

Problem

Multiplex quantitative PCR (QPCR) is a demanding application that requires extensive optimization of reagents, which is impractical for most laboratories.

Solution

The Brilliant Multiplex QPCR Master Mix provides sensitive real-time amplification of up to four targets in a single reaction. This master mix allows the use of internal controls to provide normalization within each reaction, while saving time and reagent cost.

Maximize Quantification Accuracy

Real-time PCR can provide both absolute and relative quantification during the exponential phase of amplification. To achieve accurate quantification using either method, however, the use of an internal control is required for normalization. Multiplex QPCR allows you to amplify one or more targets of interest along with your internal control in the same reaction by using a probe with a different fluorophore for the detection of each amplicon. Unlike conventional QPCR reagents, which can sometimes amplify up to two targets, our new Brilliant multiplex QPCR master mix is specifically optimized to amplify up to four targets in one reaction. This master mix allows you to normalize your results with internal controls, providing the most accurate quantification possible.

Get the Most Out of Rare or Limited Samples

Rare or limited samples, such as those obtained from biopsies or laser microdissection can be especially challenging to analyze. The Brilliant multiplex QPCR master mix provides amplification of up to four targets per reaction, and each requires far less template than if performed in four separate reactions. Moreover, the sensitivity remains equivalent to that seen in singleplex (Figure 1). This holds true across multiple dilutions of a standard curve, even at very low concentrations (Table 1). Thus, you maximize use of each precious sample without sacrificing sensitivity. In addition, the Brilliant multiplex master mix provides sufficient reaction components to accurately quantify both low and high abundance targets in the same tube.

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Increase Throughput and Decrease Reagent Cost

The Brilliant multiplex QPCR master mix is a convenient 2x formulation of SureStart Taq DNA polymerase, dNTPs and an optimized multiplex buffer. Just add your template, primers and probes and go. The convenience of the master mix format and the ability to amplify up to four targets in each reaction can save you time and rapidly accelerate your throughput. In addition, you will save reagent costs when you use the Brilliant multiplex QPCR master mix compared to running each target in a single reaction.

Let Us Help You Succeed

Whether you are new to multiplexing or want to take your multiplex to the next level, our products can help. Multiplex QPCR can prove challenging. It requires careful design and optimization of primers and probes. Our new Brilliant multiplex QPCR master mix takes the guesswork out of optimizing for up to four targets per reaction. Our Technical Services team is also available to help make your multiplex QPCR successful.

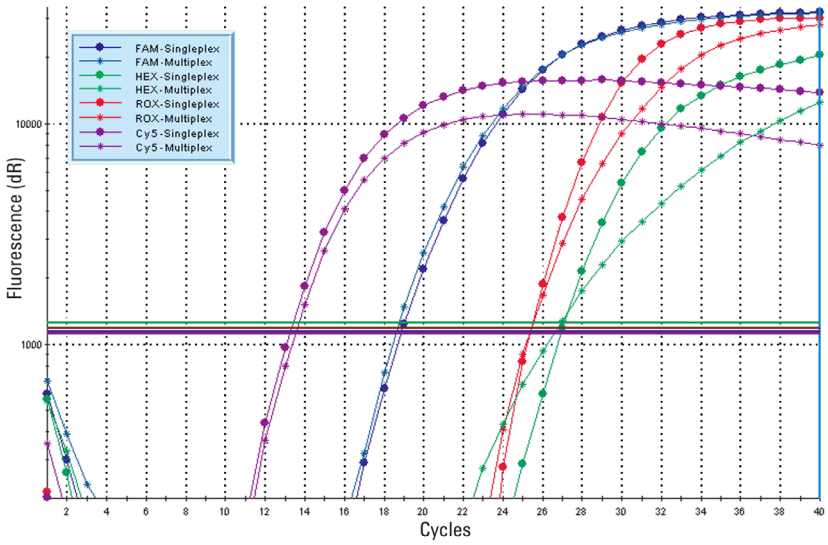

Figure 1. ***Equivalent Performance with 4-Target Multiplex***
We ran four targets—eNOS (FAM), HFE (HEX), CFTR (ROX), and Cyclophilin (Cy5)—in singleplex and multiplex using the Brilliant multiplex QPCR master mix on the Mx3000P real-time PCR system. Ct values for each target were virtually identical in the two reactions, indicating full sensitivity and performance in multiplex.

Target Concentration	FAM Singleplex	FAM Multiplex	HEX Singleplex	HEX Multiplex	ROX Singleplex	ROX Multiplex	Cy5 Singleplex	Cy5 Multiplex
40 ng	20.3	20.0	22.5	22.3	26.1	26.3	12.7	13.0
10 ng	22.3	22.1	24.4	24.4	28.1	28.3	15.1	15.3
2.5 ng	24.4	24.1	26.5	26.4	30.1	30.1	17.0	17.2
0.625 ng	26.3	26.3	28.5	28.6	32.2	32.3	19.0	19.2
0.156 ng	28.5	28.5	30.9	30.2	34.2	34.3	21.1	21.4
NTC	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct
Efficiency (%)	98.1	92.1	93.4	99.5	98.0	99.4	95 9	5.3
Slope	–3.368	–3.527	–3.49	–3.333	–3.37	–3.333	–3.44	–3.44
R^2	1.000	1.000	0.998	0.997	1.000	0.999	1.000	0.999

Table 1. ***Standard Curve for 4-Target Multiplex***
We performed four-fold serial dilutions for each of the four targets in Figure 1. We analyzed each target in both singleplex and multiplex, and prepared standard curves for each using the Brilliant multiplex QPCR master mix on the Mx3000P real-time PCR system. A comparison of Ct, efficiency, and R^2 values for each target illustrates the excellent performance characteristics of the Brilliant multiplex master mix. Each Ct value is an average of triplicate reactions.

Brilliant Multiplex QPCR Master Mix

	Contents	Catalog
Brilliant Multiplex QPCR Master Mix	100 reactions	600553

www.agilent.com/genomics/BrilliantMultiplex

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