Product Testing Report

G891/G892. Blastaq™ 2X qPCR MasterMix

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Product Efficiency and Sensitivity

Purpose

To compare the performance of Blastaq™ 2X qPCR MasterMix to two other leading brand competitor products.

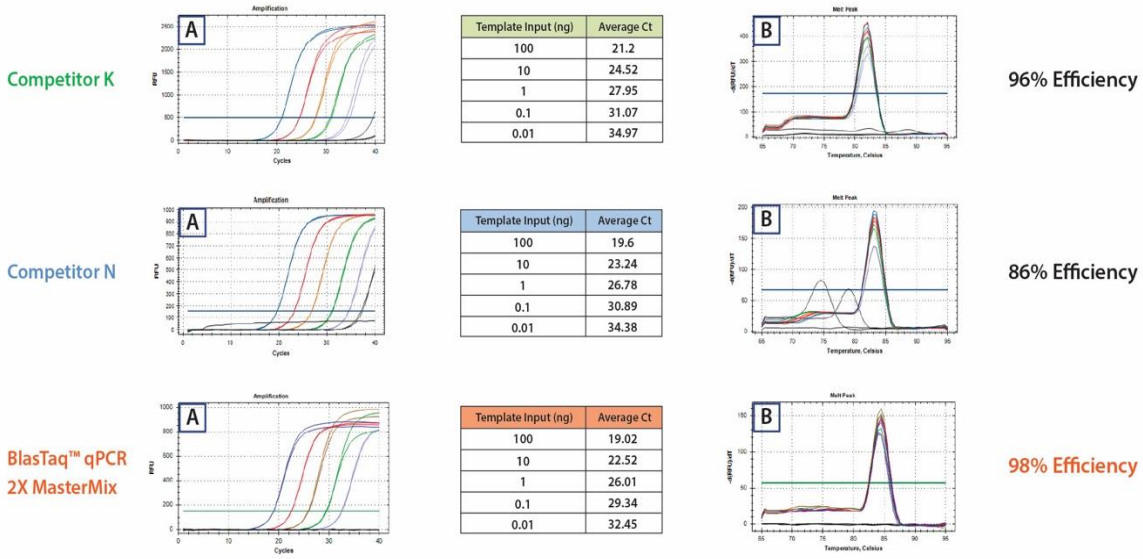
Method

A GAPDH 10-fold dilution expression assay was conducted using HEK293 cells as the template, following the respective manufacturer protocols and thermocycling conditions. The maximum template input was set at 100 ng, while the minimum was 10 pg. Negative template control (NTCs) reactions received an identical volume of nuclease-free water instead of the template. Each dilution was performed in duplicate.

After data collection, the melting curve peaks were recorded and assessed. A standard curve was then constructed using the cycle threshold (Ct) value as a function of DNA template input. The qPCR efficiency was calculated from the slope of the standard curve:

$$E = -1 + 10^{\left(-\frac{1}{slope}\right)}$$

Figure 1 - BlasTaq™ qPCR 2X MasterMix demonstrates higher efficiency, sensitivity, and is free of non-specific amplification when compared to two competitor products from major manufacturers.



Result

Blastaq™ 2X qPCR MasterMix demonstrated an efficiency rate of 98%, surpassing Competitors K and N, which had lower efficiency rates of 96% and 86% respectively. Additionally, the earlier Ct values observed with identical template inputs suggest higher sensitivity in detecting the target sequence. As shown in Figure 1, Blastaq™ 2X qPCR MasterMix consistently exhibited earlier amplification curves, particularly in comparison to Competitor K.

The melting curve analysis predicted a single strong peak at 84.5 °C. This was observed for both Blastaq™ 2X qPCR MasterMix and Competitor K. However, Competitor N displayed additional peaks at lower melting temperatures, likely indicating the presence of primer-dimer formations and/or non-specific amplification.

Conclusion

Blastaq™ 2X qPCR MasterMix is more robust and sensitive compared to two other leading brands. Moreover, it is free of non-specific amplification signals.

Detection of Low-Expression Targets and Reproducibility

Purpose

To compare the performance of Blastaq™ 2X qPCR MasterMix to two other leading brand competitor products.

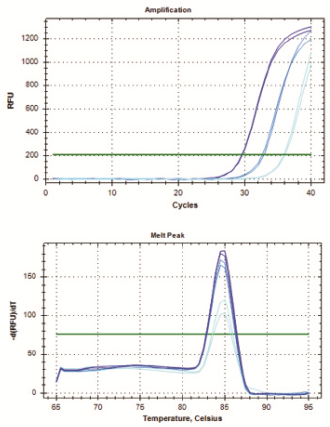
Method

Two low-expression target genes, designated “rat1” and “rat2,” were selected for analysis. Adult rat cDNA was used as the template, with starting concentrations ranging from 75 ng to 0.75 ng. Competitor product reactions were prepared according to the manufacturer’s protocols and thermocycling conditions. Negative template control (NTCs) reactions received an equivalent volume of nuclease-free water instead of the template. Each dilution was performed in duplicate. After data collection, the melting curve peaks were recorded and evaluated.

Figure 2 - BlasTaq™ qPCR 2X MasterMix offers superior reproducibility and sensitivity compared to competitors, making it ideal for analyzing non-housekeeping genes and low-expression targets. Rat genes 1 (A) and 2 (B) were amplified using adult rat cDNA as the template, with each dilution performed in duplicates. Single melting peaks were observed at 84.5°C (A) and 82°C (B). Reactions with competitor products were prepared according to the manufacturers' protocols.

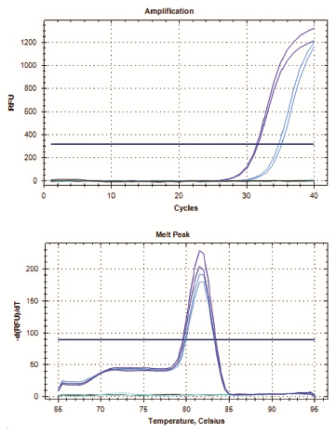
A. Rat gene 1

BlasTaq™ qPCR 2X MasterMix



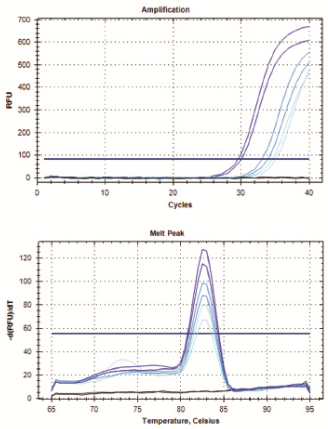
Template Input (ng)	Average Ct
75	29.55
7.5	32.69
0.75	35.93

Competitor K MasterMix



Template Input (ng)	Average Ct
75	31.56
7.5	34.93
0.75	-

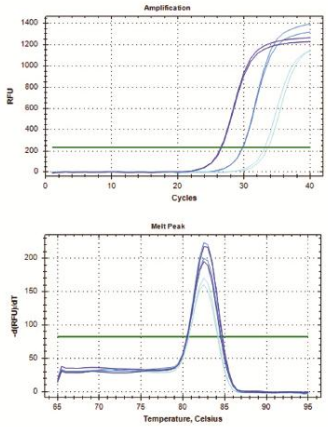
Competitor N MasterMix



Template Input (ng)	Average Ct
75	29.80
7.5	33.52
0.75	34.83

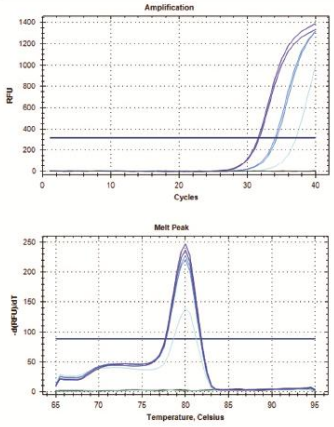
B. Rat gene 2

BlasTaq™ qPCR 2X MasterMix



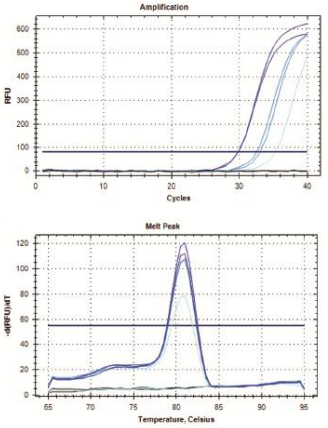
Template Input (ng)	Average Ct
75	26.54
7.5	29.82
0.75	33.40

Competitor K MasterMix



Template Input (ng)	Average Ct
75	31.63
7.5	34.17
0.75	37.13

Competitor N MasterMix



Template Input (ng)	Average Ct
75	29.82
7.5	32.81
0.75	35.27

Result

Low-expression targets typically exhibit late Ct values and can present challenges with reproducibility. Unlike Competitor K (Figure 2A), Blastaq™ 2X qPCR MasterMix demonstrates enhanced sensitivity by effectively amplifying all three template inputs for both gene targets (Figures 2A and 2B). Specifically, Blastaq™ 2X qPCR MasterMix results in the earliest Ct values overall, except for the rat 1 gene at 0.75 ng. Although Competitor N shows a lower average Ct value (34.83) compared to Blastaq™ 2X qPCR MasterMix (35.93), it produces similar Ct values for 1.5 ng and 0.15 ng inputs, which may indicate reduced sensitivity. The results from Competitor N make it difficult to differentiate between higher and lower input concentrations, suggesting limited assay performance in distinguishing varying levels of target nucleic acid.

Furthermore, Blastaq™ 2X qPCR MasterMix produced consistent results in duplicates, whereas Competitors N and K failed to achieve similar consistency at the lowest input template of 0.75 ng.

Reproducibility was assessed based on the overlap of amplification curves for the same concentration. Competitor N showed a noticeable degree of deviation at 7.5 ng for both rat 1 and rat 2 gene targets. In contrast, Blastaq™ 2X qPCR MasterMix exhibited tight and nearly identical replicates.

Conclusion

Blastaq™ 2X qPCR MasterMix is better suited for assays involving low-expression targets that require greater sensitivity and reproducibility compared to two other major manufacturers.