

Multiplex, low-volume one-step RT-qPCR for gene expression analysis using the IntelliQube® and BHQ® probes

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One-step reverse transcription-PCR (RT-PCR) is widely used for gene expression analysis, RNA virus detection, and routine RNA quantification experiments.

In this study, we evaluated the IntelliQube® real-time PCR instrument for multiplex gene expression analysis using commercially available RNA from human liver, lung, and kidney tissue, a one-step RT-PCR master mix, and BHQ® probe-based assays from LGC Biosearch Technologies. BHQ probes and primers were designed to target the mucin 1 (MUC1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the TATA-box binding protein (TBP) in a triplex PCR reaction. The IntelliQube utilizes the Array Tape® consumable and combines liquid handling with real-time qPCR analysis in 1.6 µL reaction volumes.

Using TBP and GAPDH as reference genes, it was determined that Muc1 was upregulated in kidney 143-fold and lung 297-fold in comparison to liver, which correlated with previously published data. Using a three way ANOVA, it was shown that there was no significant difference in results between singleplex and triplex reaction formats, or between multiple runs on the IntelliQube.

This study demonstrates the ability of this instrument and the associated Array Tape technology to successfully multiplex BHQ qPCR assays in a one-step RT-PCR format for gene expression studies, without compromising data quality. Combined with the automated inline process and economic benefits of Array Tape, the IntelliQube and associated BHQ probe chemistry may prove to be a very useful platform for multiplex qPCR applications in Human Genetics.