Application Note

Higher sensitivity of qPCR reactions with BRAND 384-well PCR plates

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Introduction

In many laboratories real-time quantitative PCR (RT-qPCR) has become a standard technique to correlate phenotypic observations not only with altered protein expression data but also with quantitative changes on a transcriptional level. The quality of results obtained e.g. by RT-qPCR depends among others on adequate primers for reverse transcriptase and qPCR reaction, proper RNA sample preparation and well-defined reference genes. However, the best experimental design will give poor results if external factors like malfunction of thermocyclers and inadequate PCR-vessels disturb the reaction.

Here we show for example that white 384-well PCR-plates from BRAND amplify signals much better compared with the plate of another well-known manufacturer plates in the market.

Material and Methods

Murine hippocampi were homogenized in peqGOLD RNAPure™ buffer (PeqLab) with TissueLyser (Qiagen). Total RNA was extracted using RNeasy Kit (Qiagen). cDNA was synthesized from 1 µg total RNA using iScript™ cDNA Synthesis Kit (Bio-Rad).

For RT-qPCR the following reaction was set up:

0,5 µl Primer 5 µM each
5 µl SYBR®select (2X)
1 µl cDNA
3 µl H_2O

10 µl total

SYBR®Green based gene expression reactions were loaded in triplicates in white 348-well PCR plates from BRAND (781358) and a competitor. Plates were sealed with qPCR sealing films from BRAND (#781391). PCR was performed in the CFX384TM real-time PCR machine (Bio-Rad).

Results

In the two different white 384-well PCR-plates none of the PCR-reactions failed. However, signal intensity was much stronger in the BRAND plates when compared to the competitor.

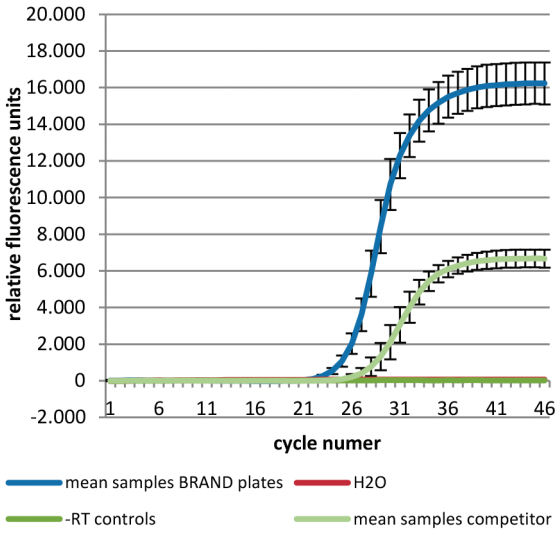

Figure: Data show mean and standard deviation of 384 RT-qPCR results per plate.

Conclusion

RT-qPCR runs more efficient in the white 384-well PCR-plates from BRAND in comparison with the plate from another well-known manufacturer as indicated by the slope and the plateau of the two different curves. This might be the result of optimized thin walled wells leading to a fast and homogenous thermal transfer and by the use of raw materials from which less PCR-inhibiting substances could be released.

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