Performance comparison of QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems

Product information

The Applied Biosystems™ QuantStudio™ 6 and 7 Pro Real-Time PCR Systems provide a transformative qPCR workflow experience to researchers. These instruments offer innovative technology, incorporating smart workflow features such as hands-free operation with facial login, voice commands, and an RFID reader for ultimate ease of use, while also providing the best features of the current Applied Biosystems™ QuantStudio™ 6 and 7 Flex Real-Time PCR Systems, in a smaller footprint. Smart workflow enhancements increase speed to results, allowing users to stay connected for monitoring experiments and analyzing or sharing data remotely at any time using Connect, our cloud-based platform. These enhancements also enable improved uptime through remote serviceability. In addition, the Thermo Scientific™ Orbitor™ RS2 Microplate Mover is available for the QuantStudio 7 Pro Real-Time PCR System to support high-throughput workflows. Both systems provide a large capacitive touchscreen interface and simplified workflow to help reduce user errors. These systems are designed to offer high quality, excellent reliability, and an optimal user experience for researchers who want to work smarter in the lab.

Abstract

The purpose of this paper is to show researchers how to effectively compare different qPCR platforms and to demonstrate the equivalency of relative gene expression data obtained from the Applied Biosystems™ 7900HT, QuantStudio 7 Flex, and QuantStudio 7 Pro Real-Time PCR Systems. Here we demonstrate that similar results are obtained with all three instruments in terms of relative gene expression, detection sensitivity, and PCR performance.

Introduction

Great advancements in real-time PCR instrumentation have been made over the past decades since Applied Biosystems (now part of Thermo Fisher Scientific) introduced the first commercial system, the ABI Prism 7700 Sequence Detection System, in 1996. Many users wish to take advantage of new capabilities and upgrade to more modern instruments but want to make sure they can rely on the new platforms to give them similar results. Therefore, we have undertaken a simple type of experiment to elucidate some best practices for analysis while demonstrating equivalent performance among the three qPCR platforms. This is meant as a useful guide for most researchers. In this study, gene expression of some representative biomarkers is compared across instrument types. Gene expression assays were chosen because they are a common qPCR application that is optimal to test the features of the platforms. However, the lessons learned in this study can be applied to most other types of applications, such as genotyping and presence/absence experiments.

Importantly, this experimental design does not compare primary analysis results (C_t or C_q values). This is because different platforms are not necessarily expected to have the same C_q values. Optical designs of different platforms can cause different C_q values for the same reagents, and that is normal. Therefore, we recommend comparing relative C_q values, as demonstrated in this study.

ThermoFisher SCIENTIFIC

Platform comparison results

Gene expression study

To demonstrate equivalency of relative gene expression results from data generated on the QuantStudio 7 Pro instrument to those of the QuantStudio 7 Flex and 7900HT instruments, gene expression assays for *GFAP*, *ALB*, and *KRT1* (FAM™ dye, MGB probe) and an endogenous control assay for *PPIA* (VIC™ dye, QSY™ probe, primer-limited) were run on each platform. Targets were amplified using Applied Biosystems™ TaqMan® Fast Advanced Master Mix (20 µL reactions) with cDNA generated from universal human reference (UHR) RNA, total liver RNA, and total brain RNA at inputs totaling 50 ng per reaction with 8 replicates and 8 no-template control (NTC) reactions. This relatively high input was selected to ensure sufficient amplification of each target where there was a wide range of expression across the tissues tested. Three plates were set up simultaneously and run on the QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems using 96-well, 0.2 mL blocks. On the QuantStudio 7 Pro and QuantStudio 7 Flex platforms, reactions were run in duplex with the endogenous control. Initially, 7900HT reactions were also run in duplex but due to spectral cross-talk affecting the *PPIA* signal on the predecessor 7900HT software, the plates were re-run in simplex with biomarker assays and endogenous control in separate wells. Thermal cycling times and temperatures were held constant, but default ramp rates for each instrument were used.

The qPCR data were imported to Connect and analyzed using the Relative Quantitation (RQ) app (Figure 1). Relative expression was calculated using UHR RNA as the reference sample and *PPIA* as the endogenous control. A C_q cutoff of 38 was used. The default settings of auto threshold and auto baseline were used for analysis. Connect allows analysis of data from all three platforms using a common analysis algorithm, allowing for a direct comparison of results.

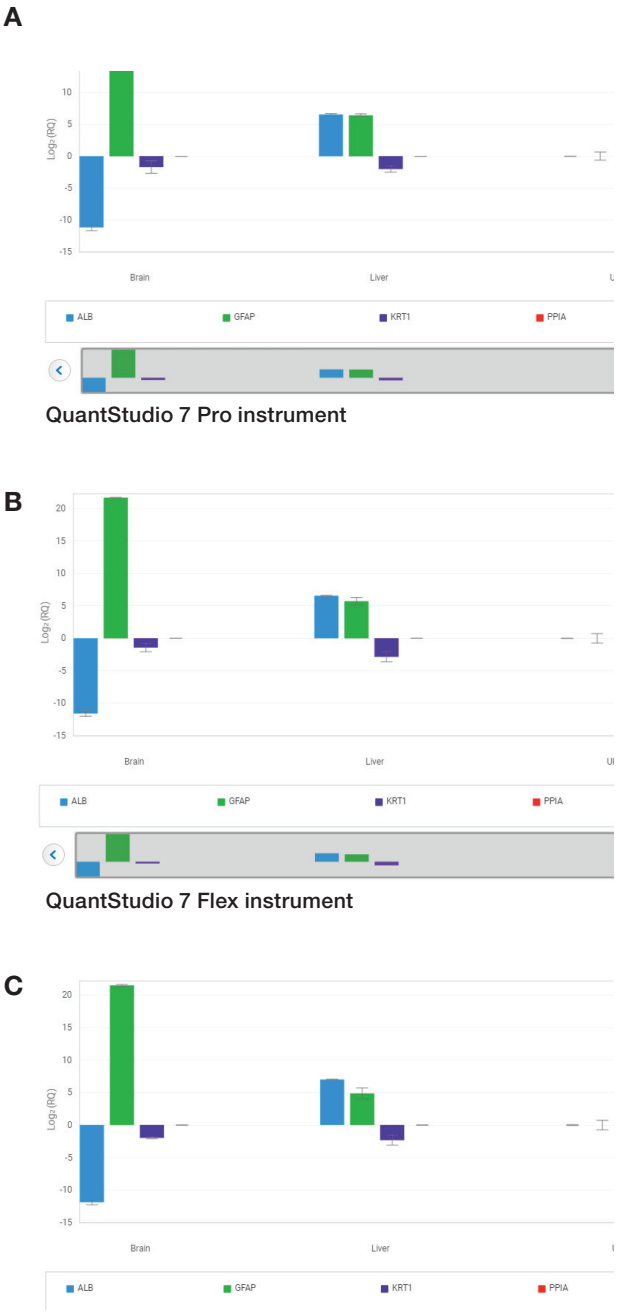


Figure 1. Comparison of gene expression data. Data are relative quantitation graphs from the **(A)** QuantStudio 7 Pro, **(B)** QuantStudio 7 Flex, and **(C)** 7900HT Real Time PCR Systems, exported from Connect, where $\log_2$ RQ values for each assay are plotted against tissue type. Although the RQ values from each instrument are not identical, the data is consistent, in that all gene expression assays demonstrate equivalent patterns of higher or lower expression in each of the tissues tested. *ALB* (blue) shows lower expression in brain and higher expression in liver, relative to UHR, across the 3 platforms. *GFAP* (green) demonstrates higher expression, while *KRT1* (purple) shows lower expression in both brain and liver, relative to UHR. In all cases, the fold-differences were in the same order of magnitude across the three platforms.

When a common software is not being used in comparing data (RQ) between different platforms (instrument models), it is advisable to use a manual threshold set separately for each platform. Specifically, for any instrument type, the threshold should be set manually in the middle of the exponential growth region of the amplification plots. This will allow comparison of dC_q values between platforms without the influence of each platform's unique optical profile. For example, a threshold of 0.2 that is in the middle of the exponential growth region of curves on one platform may be too low or too high on another platform. It is important to note that it is common to observe different C_q values when comparing data between different platforms. This is not relevant provided the C_q differences (dC_q) between the reference and the test samples remain consistent between instrument models (Figure 2). Differences can be minimized by analyzing on Connect.

Figure 2. Comparison of ddC_q data between QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems. Data show equivalent relative expression level of *ALB*, *GFAP*, and *KRT1* in the cDNA of total brain RNA and total liver RNA (data generated in Microsoft™ Excel™ software).

We found a high degree of agreement between the three platforms. Although the values of the fold change measurements were not identical in these comparisons, the pattern of low or high expression in each tissue, relative to UHR RNA, was the same for each gene expression assay tested.

Detection sensitivity

To demonstrate the equivalent discrimination sensitivity of the QuantStudio 7 Pro instrument to the QuantStudio 7 Flex and 7900HT instruments in the detection of small fold changes, samples with 2-fold and 1.5-fold differences in input were assessed. Total liver cDNA at inputs of 5 ng, 3.33 ng, and 2.5 ng were added to 20 µL reactions. As with the previous gene expression study, reactions on the QuantStudio 7 Pro and QuantStudio 7 Flex instruments were run in duplex with a *PPIA* endogenous control. Each input was run with 16 replicates, along with 4 NTC reactions. As with the gene expression study, the reactions on the 7900HT instrument were again run in simplex due to spectral cross-talk, with 14 replicates per input and 4 NTC reactions. Thermal cycling times and temperatures were held constant between the different platforms, but default ramp rates for each instrument were used.

Runs were analyzed using their respective platform software, with auto threshold and baseline settings, then exported and analyzed using JMP™ v13 statistical software, (Table 1). For this analysis, because the dC_q is compared within a single target, auto-threshold C_q calculation is appropriate. To demonstrate discrimination of a 2-fold change, a Student's *t*-test was performed for the 5 ng and 2.5 ng inputs, to test for significant differences at 99.9% confidence. Analysis was repeated for the 5 ng and 3.33 ng inputs, to demonstrate a 1.5-fold change. All three instruments were found to exhibit equivalent ability to detect 2-fold and 1.5-fold changes, for both *ALB* and *PPIA* targets, with a 99.9% confidence (all *P* values of <0.0001). Representative amplification data generated with the QuantStudio 7 Pro Real-Time PCR System are shown in Figure 3.

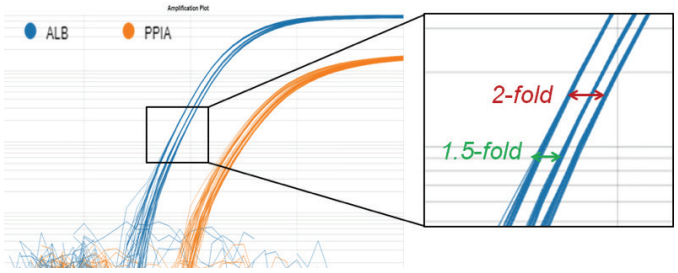


Figure 3. An example of detection sensitivity on the QuantStudio 7 Pro Real Time PCR System. Clustering of amplification curves demonstrate distinct 2- and 1.5-fold differences.

Table 1. Amplification C_q data from detection sensitivity comparison of small fold changes in cDNA quantity performed on the QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems. All three instruments were found to demonstrate equivalent ability to detect 2-fold and 1.5-fold changes, for both *ALB* and *PPIA* targets, with a 99.9% confidence (all *P* values of <0.0001).

		7900HT		QuantStudio 7 Flex		QuantStudio 7 Pro	
	cDNA quantity	C_q mean	C_q SD	C_q mean	C_q SD	C_q mean	C_q SD
ALB	2.5 ng/µL	21.82	0.06	21.64	0.02	21.64	0.03
	3.33 ng/µL	21.35	0.06	21.27	0.02	21.25	0.03
	5 ng/µL	20.86	0.05	20.72	0.02	20.67	0.02
PPIA	2.5 ng/µL	26.97	0.08	25.86	0.09	26.14	0.11
	3.33 ng/µL	26.50	0.10	25.46	0.12	25.76	0.10
	5 ng/µL	25.86	0.07	24.81	0.12	25.07	0.14

Dilution series

A dilution series was performed to demonstrate the equivalence of the QuantStudio 7 Pro instrument to the QuantStudio 7 Flex and 7900HT instruments in linear detection. A 6-log dilution series of 0.0005 ng to 500 ng of cDNA per 20 µL reaction was used for assay targets *GFAP* (with cDNA from total brain RNA), *ALB* (with cDNA from total liver RNA), and *KRT1* (with cDNA from UHR RNA) in triplicate with triplicate NTCs (Figure 4). As with the previous gene expression study, reactions on the QuantStudio 7 Pro and QuantStudio 7 Flex Real-Time PCR Systems were run in duplex with a *PPIA* endogenous control, while the reactions on the 7900HT Real-Time PCR System were run in simplex due to spectral cross-talk. Thermal cycling times and temperatures were held constant between the different platforms, but default ramp rates for each instrument were used.

Runs were analyzed for each assay using their respective platform software, with auto threshold and baseline settings, then exported and analyzed using JMP v13 statistical software. A linear fit was generated for each assay (Figure 5). Slope, PCR efficiency, and R^2 values were calculated and compared between the three instruments. Linear fit was calculated for 6 logarithmic units for *ALB*, *GFAP*, and *PPIA* targets. *KRT1* was a comparatively low-expressing target; the higher dilution points were outside the linear range for the assay, so linear fit was calculated from the first four dilutions (3 logarithmic units).

An R^2 value of >0.98 and PCR efficiency of 90–110% were used as benchmark metrics. For this study we were primarily focused on equivalency of these metrics between the three platforms tested. The results are summarized in Table 2. For *ALB*, *GFAP*, and *PPIA* across platforms, R^2 values were 1.00. PCR efficiency between platforms was within ±5%. *KRT1* demonstrated higher variation, with R^2 of 0.99 for the QuantStudio 7 Pro and 7900HT instruments, and 0.97 for the QuantStudio 7 Flex instrument, and PCR efficiency within ±7% between platforms. As stated, *KRT1* expression levels were comparatively low in this dilution series. Under such circumstances, it is not unexpected to see higher variation in these metrics. Overall, results of this comparison demonstrate that all three platforms exhibit similar PCR performance.

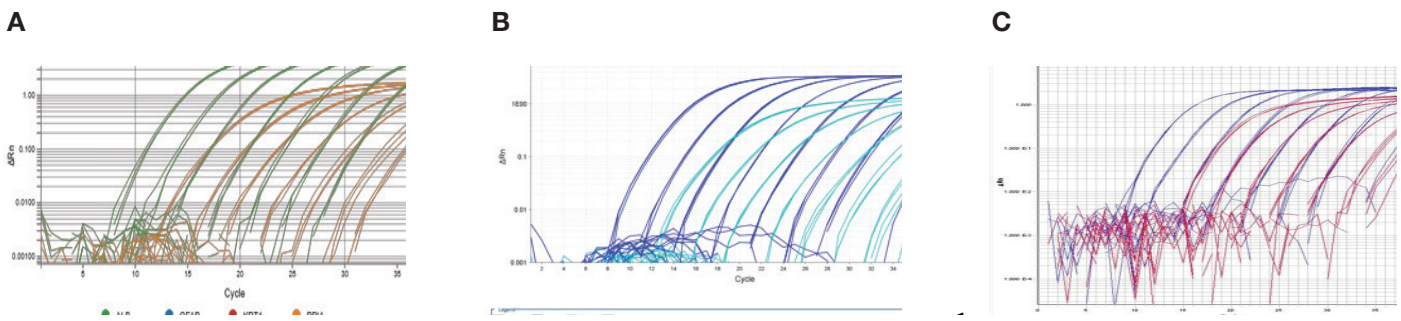
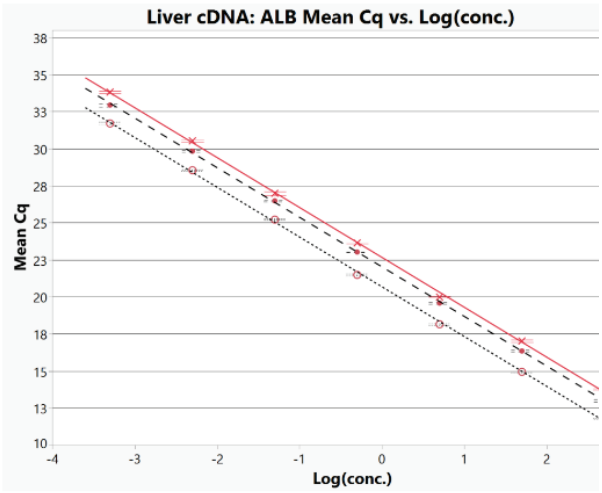
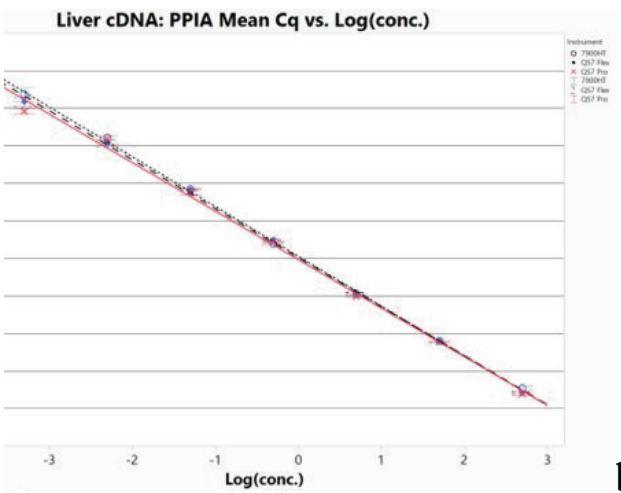


Figure 4. Amplification plots of cDNA dilution series. Data were produced on **(A)** QuantStudio 7 Pro (*ALB*, green; *PPIA*, orange), **(B)** QuantStudio 7 Flex (*ALB*, blue; *PPIA*, teal), and **(C)** 7900HT (*ALB*, blue; *PPIA*, red) Real-Time PCR Systems for assay targets *ALB* and *PPIA*.

A



B



. Data show targets **(A)** *ALB* and **(B)** *PPIA* on QuantStudio 7 Pro (red line), QuantStudio d line) Real-Time PCR Systems. Equivalence with an R^2 of 1 was demonstrated across all *PPIA*.

(b) on QuantStudio™ 7 Pro (red line),

Table 2. Linear regression analysis of targets *GFAP* from total brain cDNA, *ALB* from total liver cDNA, *KRT1* from UHR cDNA, and *PPIA* from all tissues on QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems. An equivalent R^2 of 1 with a PCR efficiency within 5% for each of the *ALB*, *GFAP*, and *PPIA* assays, and an R^2 of 0.97–0.99 with an efficiency within 7% for *KRT1* was demonstrated between instruments.

		7900HT			QuantStudio 7 Flex			QuantStudio 7 Pro		
Sample type	**Gene**	**Slope**	**R^2**	**Efficiency**	**Slope**	**R^2**	**Efficiency**	**Slope**	**R^2**	**Efficiency**
Brain	*GFAP*	–3.37	1.00	97.88	–3.40	1.00	96.83	–3.39	1.00	97.40
	PPIA	–3.40	1.00	96.85	–3.41	1.00	96.62	–3.43	1.00	95.57
Liver	*ALB*	–3.36	1.00	98.54	–3.35	1.00	99.01	–3.37	1.00	98.19
	PPIA	–3.31	1.00	100.32	–3.26	1.00	102.55	–3.23	1.00	104.00
UHR	*KRT1*	–3.32	0.99	100.21	–3.29	0.97	101.25	–316	0.99	107.30
	PPIA	–3.31	1.00	100.56	–3.26	1.00	102.84	–3.26	1.00	102.52

Platform comparison conclusions

Similar results were obtained with QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems in terms of biomarker relative expression, discrimination, and PCR performance. Relative gene expression studies demonstrated equivalency of relative gene expression results from data generated on the QuantStudio 7 Pro instrument to those of the QuantStudio 7 Flex and 7900HT instruments using three biomarker assays. Although the RQ values from each instrument were not identical, the data were consistent between instruments. Comparison of ddC_q data between QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems demonstrated equivalent relative quantitation of *GFAP*, *ALB*, and *KRT1* expression in cDNA of UHR RNA, total liver RNA, and total brain RNA.

Equivalency of the QuantStudio 7 Pro instrument to the QuantStudio 7 Flex and 7900HT instruments was also demonstrated with a detection sensitivity comparison. 2-fold and 1.5-fold differences in input were demonstrated for both *ALB* and *PPIA* targets, with a 99.9% confidence. Linear regression analysis of targets *GFAP* from total brain cDNA, *ALB* from total liver cDNA, *KRT1* from UHR cDNA, and *PPIA* from all tissues performed on QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems demonstrated equivalent R^2 values of 1 with a PCR efficiency within 5% for each of the *ALB*, *GFAP*, and *PPIA* assays and an R^2 value of 0.97–0.99 with an efficiency within 7% for *KRT1*.

User recommendations

To provide the best user experience, we recommend the following practices in comparing data across instrument platforms:

- Analyze data using Connect to standardize analysis algorithms and minimize variation
- Set up identical plates (reagents and samples) and run in parallel on different instruments
- It may be advisable to use manual rather than automatic threshold in order to minimize differences caused by different analysis algorithms
- Compare results (ddC$_q$, RQ, R^2, dynamic range, or genotyping call) rather than raw C$_q$, which can vary
- Ensure that your assays are well-qualified before conducting a comparison

Using these methods, we demonstrated equivalency of biomarker expression data obtained from the 7900HT, QuantStudio 7 Flex, and QuantStudio 7 Pro Real-Time PCR Systems. Researchers can use similar methodology and best practices to inform their own transition studies, maintaining the high quality and fast pace of data acquisition that is expected of real-time PCR.

Platform comparison methods

All runs were performed using a standard cycling profile and ramp rates applied across all platforms (Table 3) and Applied Biosystems™ TaqMan® Gene Expression Assays for *GFAP*, *ALB*, and *KRT1* (Table 4). The following instrument software versions were used for analysis: QuantStudio Real-Time PCR Software v1.3, QuantStudio 7 Pro Design and Analysis Software v1 (prerelease), 7900HT SDS 2.4.1, and Connect RQ v2 (prerelease).

cDNA generation

cDNA was generated from Universal Reference RNA (Agilent, Cat. No. 740000), Invitrogen™ Human Brain Total RNA (Cat. No. AM7962), and Invitrogen™ Human Liver Total RNA (Cat. No. AM7960) using Applied Biosystems™ High Capacity cDNA Reverse Transcription Kit (Cat. No. 4368813). RT reactions consisted of 10 µg of total RNA in a 20 µL reaction. This unusually high concentration for an RT reaction was used to generate cDNA stocks at greater concentrations to accommodate dilution series inputs. The plate was run on an Applied Biosystems™ ProFlex™ PCR System with the standard protocol per the High Capacity cDNA Reverse Transcription Kit user manual with the exception of a 12 hr 37˚C incubation and an additional 10 min 85˚C incubation for degradation of remnant RNA. UHR cDNA, total liver cDNA, and total brain cDNA were recovered and stored at –20°C.

Table 3. Cycling profile for QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems using TaqMan Fast Advanced Master Mix (Cat. No. 4444963).

Block	Mode	Reporters	Reaction volume	Cycles	Temperature	Time
96-well standard	96-well 0.2 mL block	FAM, VIC	20 µL	Hold	50°C	2 minutes
				Hold	95°C	20 seconds
				40	95°C	3 seconds
					60°C	30 seconds

Table 4. TaqMan Assays used for equivalency comparison of gene expression detection between QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems.

Assay name	Gene
Hs00910225_m1	*ALB*
Hs00196158_m1	*KRT1*
Hs00266705_g1	*GFAP*
Human PPIA (Cyclophilin A) Endogenous Control (Cat. No. 4326316E)	*PPIA*

Gene expression assays

cDNA generated from UHR RNA, total liver RNA, and total brain RNA was amplified using TaqMan Gene Expression Assays for *GFAP*, *KRT1*, and *ALB* (Table 4). Inputs totaled 50 ng per 20 µL reaction in TaqMan Fast Advanced Master Mix, with 8 replicates and 8 NTCs. Three plates were set up simultaneously and run on the QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems using 0.2 mL standard blocks. On the QuantStudio 7 Pro and QuantStudio 7 Flex instruments, reactions were run in duplex with the endogenous control. Reactions on the 7900HT instrument were initially run in duplex. However, analysis demonstrated spectral cross-talk with the predecessor instrument software. Therefore, the 7900HT reactions were subsequently run in simplex with gene expression assays and endogenous control run in separate wells (data shown in this report). For each instrument, thermal cycling times and temperatures were held constant, but default ramp rates for each instrument were used (Table 3).

Detection sensitivity

cDNA sample inputs totaling 5 ng, 3.33 ng, and 2.5 ng of total liver cDNA per 20 µL reaction in TaqMan Fast Advanced Master Mix were used for detection sensitivity comparison. As with the biomarker study, reactions on the QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems were originally run in duplex with a *PPIA* endogenous control for the *ALB* biomarker assay (Table 4). Duplex reactions were performed with 16 replicates of each input, along with 4 NTC reactions. Due to spectral cross-talk on the 7900HT instrument, the 7900HT reactions were subsequently run in simplex, with 14 replicates of each input and 3 NTC reactions per assay to accommodate plate setup design (data shown in this report). Thermal cycling times and temperatures were held constant between the different platforms, but default ramp rates for each instrument were used (Table 3).

Dilution series

A 6-log dilution series of 0.0005 ng to 500 ng of cDNA per 20 µL reaction in TaqMan Fast Advanced Master Mix was used for assay targets *GFAP* (with cDNA from brain), *ALB* (with cDNA from liver), and *KRT1* (with cDNA from UHR) (Table 5) in triplicate with triplicate NTCs. As with the biomarker study, reactions on the QuantStudio 7 Pro and QuantStudio 7 Flex instruments were run in duplex with a *PPIA* endogenous control, while the reactions on the 7900HT instrument were run in simplex due to spectral cross-talk. Thermal cycling times and temperatures were held constant between the different platforms, but default ramp rates for each instrument were used (Table 3).

Table 5. cDNA sources per assay target used in dilution series comparison of QuantStudio 7 Pro, QuantStudio 7 Flex, and 7900HT Real-Time PCR Systems.

Assay name	Gene	cDNA source
Hs00910225_m1	*ALB*	Total liver RNA
Hs00196158_m1	*KRT1*	UHR RNA
Hs00266705_g1	*GFAP*	Total brain RNA

applied
biosystems

Find out more at **thermofisher.com/quantstudiopro**

ThermoFisher
SCIENTIFIC