



SmartStart Orientation: Absolute Quantification Hands-On Training

for use with:

QuantStudio™ 6 Pro and QuantStudio™ 7 Pro Real-Time PCR Systems

This guide describes the main workflow steps involved in an absolute quantification experiment. At the end of this training users will be able to set up an absolute quantification experiment applying best lab practice recommendations and utilize key features of the instrument software, including setting up an experimental run and performing data analysis.

Table 1: Reagents included with QuantStudio™ 6 Pro and QuantStudio™ 7 Pro Real-Time PCR Systems SmartStart Orientation (TRN00303).

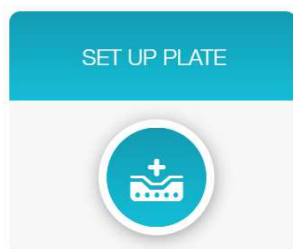
Component	Cat. No.	Amount	Storage
TaqMan® Control Genomic DNA (human) 10ng/uL	4312660	1 tube	-20°C
Taqman RNase P Assay	4312660	1 tube	
TaqMan Fast Advanced Master Mix	4444557	1 tube	4°C
Nuclease-Free Water (not DEPC-Treated)	AM9938	1 bottle	Room Temperature

Table 2: Reagents *not* included with QuantStudio™ 6 Pro and QuantStudio™ 7 Pro Real-Time PCR Systems SmartStart Orientation (TRN00303). These reagents are required for the training and should be supplied by the customer.

Component
1.5-mL RNase and DNase free Microcentrifuge tubes
Minicentrifuge
Vortex
Micropipettes (to pipette from 5ul to 500ul)
Aerosol-resistant barrier pipette tips
Plate centrifuge – to spin the plates before loading into the instrument.
Optical reaction plate compatible with Real Time PCR instrument configuration
Optical adhesive film and applicator
Real-time PCR plate holder
1.5 ml tube rack and sharpies
Disposable gloves

Step 1: Setup the experiment in Design and Analysis Software

1. Launch Design and Analysis Software and click on **SET UP PLATE**.



2. In the Templates tab, check the appropriate selections in the Properties panel. Then select the correct Template File.
 - a. Be sure that the correct block and instrument type are selected for the instrument configuration.
 - b. Taqman Fast Advanced Master Mix cycles in Fast mode for all block types.
 - c. Select Standard Curve for Analysis.
3. If necessary, change the sample volume in the Run Method to 20uL.

Sample Volume

4. Use the default thermal cycling parameters for this experiment.

Table 3: qPCR Thermal Cycling Conditions

qPCR Stage	Temperature (°C)	Time	Cycles
Hold	95	20 seconds	x40
PCR	95	1 second	
	60	20 seconds	

Properties**Instrument**

- ☐ QuantStudio ® 3
☐ QuantStudio ® 5
☐ QuantStudio ® 6 Pro
☐ QuantStudio ® 7 Pro

Block

- ☐ 96-Well 0.1-mL
☐ 96-Well 0.2-mL
☐ 384-Well
☐ TLDA

Run Mode

- ☒ Fast
☐ Standard

Analysis

- ☒ Standard Curve
☐ Genotyping
☐ Presence Absence
☐ Relative Quantity

5. Go to the Plate Setup tab and assign your plate map as follows:

	1	2	3	
A	10000 RNase P x	10000 RNase P x	10000 RNase P x	Enter sa
B	2500 RNase P x	2500 RNase P x	2500 RNase P x	Enter sa
C	625 RNase P x	625 RNase P x	625 RNase P x	Enter sa
D	156.25 RNase P x	156.25 RNase P x	156.25 RNase P x	Enter sa
E	39.06 RNase P x	39.06 RNase P x	39.06 RNase P x	Enter sa
F	Unknown 1 RNase P x	Unknown 1 RNase P x	Unknown 1 RNase P x	Enter sa
G	Unknown 2 RNase P x	Unknown 2 RNase P x	Unknown 2 RNase P x	Enter sa
H	NTC RNase P x	NTC RNase P x	NTC RNase P x	Enter sa

Step 2: Prepare Standard Curve Dilutions

Note: The stock concentration of TaqMan® Control genomic DNA is 10ng/uL (10,000pg/uL). Calculate the amount of gDNA needed for each of the five points on a four-fold dilution series starting with a copy number of 10,000 and using 100uL as your final volume.

- Calculate the mass of gDNA needed in the 4-fold dilution series below.

Table 4: Copy to Mass Conversions

Copy Number	Mass of RNase P (pg)	Mass of gDNA Required (pg)
10,000	3.3	33,000
2,500		8,250
625		2,062.50
156.25		515.63
39.06		128.9

- Calculate the concentration of gDNA needed to achieve the required copy numbers of interest in 5uL.

Note: We will be adding 5uL of gDNA into each qPCR reaction. The final reaction volume will be 20uL.

Table 5: Calculation to determine required concentration of gDNA.

Copy Number	Mass of gDNA Required (pg)	Reaction Volume (uL)	Final Concentration (pg/uL)
10,000	33,000	5	6,600
2,500	8,250		1,650
625	2,062.50		412.50
156.25	515.63		103.13
39.06	128.9		25.78

- Label five 1.5mL micro-centrifuge tubes as follows: **1, 2, 3, 4, and 5.**
- Pipette 34uL of nuclease free water into tube 1.
- Pipette 75uL of nuclease free water into tube 2.
- Pipette 75uL of nuclease free water into tubes 3, 4, and 5
- Pipette 66uL of the stock gDNA from the TaqMan® Control genomic DNA (10ng/uL) tube into tube 1. Vortex well.
- Pipette 25uL of the solution from tube 1 into tube 2. Vortex well.
- Pipette 25uL of the solution from tube 2 into tube 3. Vortex well.
- Pipette 25uL of the solution from tube 3 into tube 4. Vortex well.
- Pipette 25uL of the solution from tube 4 into tube 5. Vortex well.

Table 6: DNA Serial Dilution

Tube ID	Source of gDNA	Initial Concentration (pg/uL)	Volume of gDNA (uL)	Volume of Water (uL)	Final Volume (uL)	Final Concentration (pg/uL)	Resulting Copy Number of RNase P per 5uL
1	Stock	10,000	66	34	100	6600	10,000
2	Tube 1	6600	25	75	100	1650	2500
3	Tube 2	1650	25	75	100	412.5	625
4	Tube 3	412.5	25	75	100	103.13	156.25
5	Tube 4	103.13	25	75	100	25.78	39.06

Step 3: Prepare Unknown Samples

- Label two 1.5mL micro-centrifuge tubes as follows: **Unknown 1 and Unknown 2.**
- Pipette 180uL of nuclease free water into each tube.
- Pipette 20uL from tube 1 (from your DNA dilution series) into Unknown 1. Vortex well.
- Pipette 20uL from tube 4 (from your DNA dilution series) into unknown 2. Vortex well.

Table 7: Unknown Sample Dilution

Tube ID	Source of gDNA	uL of gDNA	uL of Water
Unknown 1	Tube 1	20	180
Unknown 2	Tube 4	20	180

Step 4: Setup the Absolute Quantification Reaction

1. Label one 1.5mL micro-centrifuge tube as **Master Mix**. Prepare the mix as specified in the table below.

Table 8: RNase P Master Mix

Reagent	Reaction Volume	Reactions	Final Volume
2x TaqMan Master Mix	10	29	290
20X RNase P Assay	1		29
Water	4		116
Total Volume	15		435

Note: 29 Reactions= (5 dilutions + 2 unknowns + 1 NTC) x 3 replicates + 5 extra reactions

5. Mix well and distribute 15uL of the master mix into each of the plate's wells according to the plate layout.
6. Mix well and distribute 5uL of each dilution into each of the plate's wells according to the plate layout.
7. Mix well and distribute 5uL of each unknown into each of the plate's wells according to the plate layout.
8. Add 5uL of nuclease-free water for NTCs according to the plate layout.
9. Seal the plate with an optical adhesive cover. Be sure to all edges of the seal are firmly pressed down to prevent evaporation.
10. Briefly spin the qPCR plate in a compatible centrifuge (1,200 x g for 20 seconds).

Step 5: Load the plate into the instrument.**Step 6: Save run file and start run.**