ProQuantum[™] Technical Guide USER GUIDE

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Revision	Date	Description	
B.0	3 November 2021	The manufacturer address was updated.	
A.0	6 November 2017	New manual for ProQuantum [™] Immunoassay Kits.	

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Product information

Product description

ProQuantum[™] high-sensitivity immunoassays are designed to detect and quantify target-specific proteins using qPCR as a readout. The assay has a large dynamic range with high sensitivity and uses small sample volumes. The workflow is steamlined with no wash steps. Due to the pushing the boundaries for high sensitivity, no wash, no shaking assay with minimal volumes, it is important to pay close attention to pipetting accuracy and mixing steps to achieve good results.

Contents and storage

ProQuantum[™] immunoassay kits are shipped frozen on dry ice. Upon receipt, store the kits at –20°C. Do not store at –80°C.

Each kit comes with all the reagents necessary to perform the assay.



Workflow



Prepare working plate







ProQuantum[™] Technical Guide

Methods



General guidelines

- The ProQuantum[™] immunoassay is a DNA assay, and will not perform properly if samples contain inhibitors that affect such assays (e.g., DNA ligase inhibitors).
- Wear gloves, use DNase/RNase/pyrogen free plastic ware, and practice proper DNA handling techniques.
- Use a plate seal hand tool to ensure complete adherence to avoid any evaporation or contamination.
- Use best practices with pipetting to minimize CV.
- Use a working plate to prepare reagents and a multi-channel pipette when transferring reagents to the assay plate to minimize handling time and thus, evaporation.
- Use a plate rack to hold the assay plate to avoid splashing when unsealing the adhesive seals.
- Outliers are more readily identified if the assay is run in triplicate.
- Be sure to use appropriate PCR plates that are compatible with the qPCR instrument and block type (See **thermofisher.com/plastics** for compatibility details).
- Do not vortex plates.
- Mixing of the reagents is critical to the assay. Mixing can be performed by careful pipetting up and down 10 times, or by striking the side of the plate against the palm of your hand or a stationary object like the lab bench. The strike should be hard enough to cause liquid to splash to the top of the adhesive seals and to the other side within the well.

Guidelines for handling reagents

- Centrifuge vials before pipetting to ensure the contents are at the bottom of the tube.
- **Do not thaw Ligase**. Keep Ligase at –20C or on ice at all times. Ligase is viscous. Careful with pipetting accurate amounts and not cause bubbles.
- Thaw all reagents except Ligase at room temperature.
- Larger vials like Assay Dilution Buffer may take awhile to thaw, and can be thawed in a warm water bath.
- Keep Ligase and thawed reagents on ice.
- Use a refrigerated cold block for the working plate to keep all reagents cool during preparation steps. If a cold block is not available, keep the working plate on ice.
- If any particulate matter is present in the sample, centrifuge or filter sample before performing the assay.



Guidelines for working with small volumes

- When working with small volumes, good practices are critical for the best CVs.
- Make sure pipettes are calibrated. The appropriate size pipettes are used such as 2 µL or 20 µL. When possible, the use of a multi-channel pipette can make the workflow steps easier and minimize CVs.
- Use low retention filter tips.
- The plate seals must adhere tightly, especially around the edge of the plate using the plate seal hand tool. This will prevent any evaporation as well as enabling mixing of the plate without contamination between wells.
- Use low dead volume troughs designed for smaller volumes to aid use of multi-channel pipettes.

Guidelines for preparing and calculating standard curves

- Ensure that the reconstitution and serial dilution calculation setup and steps was performed properly.
- Make sure that the reconstitution of the standard protein was correct.
- Make sure that there is adequate mixing during the serial dilution steps and that the pipette tips were disposed of at each step.
- Ensure that the standard curve range was set within the range of the extended standard curve example in the protocol.
- If desired, extend the range of the standard curve by modifying the Rate of dilution, or S1 concentration when setting up the Method in the ProQuantum[™] software.

Pro	oQuantum		Powered by Thermo	Fisher Clou	ud 🔕	Help 💻	6 9 ∪s ≛ ~
PQ	Set Up Standar	rds Set Up Plate	Assay Instructions	Import Run (Data Qualify Standards	View Results	
Set	up serial dilution						
Ca	atalog No.	A35575					
Sp	ecies/Analyte	Human IL-8	pg/ml				
Lo	ot Number		CONCENTRATION	BUFFER	10.0 µl		
St	ock Concentration	5000 pg/ml	83.333 pg/ml	75 μl S2	15 µl		
Di	lutions	7 ~	13.889 pg/ml	75 µl S	15 µl		
Ra	ate of dilution	6-fold ~	2.31481 pg/ml	75 µl S4			
Re	eplicates	Triplicates ~	0.3858 pg/ml	75 µl St	15 µl		
S1	Concentration	500 pg/ml	0.0643 pg/ml	75 µl St	5 15 µl		
w	ells for unknowns	72	0 pg/ml	75 µl (B			
C	ancel					Previous	Next

 Review the standard curve data for any outliers using the ProQuantum[™] software. The default setting for outlier detection flags any value that is outside of 70–130% standard recovery or 15% CV of the replicate data.

Required materials not provided

Product	Cat. No. [1]	
Calibrated pipettes and low retention filter tips	MLS	
96-well plate cold block	Fisher 50-589-601	
96-well assay plate and 96-well working plate	MLS	
25-mL reagent reservoir × 2	Fisher 14387071	
Microtiter plate adhesive seals × 2	Fisher 4306311	
Microtiter plate adhesive seal, optical grade	Fisher AB1170	
Plate seal hand tool	4333183	
Centrifuge with swinging bucket rotor for 96-well plates, and microcentrifuge	MLS	
96-well plate rack	Fisher 05-541-80	
RNase-free 1.5-mL microcentrifuge tube	AM12400	
Sterile 15-mL conical tube	339651	

^[1] MLS: major laboratory supplier.

Prepare reagents

Prepare serum samples

Maintain the samples at 2–8°C during handling.

- 1. Collect whole blood in a covered test tube.
- 2. Allow the collected blood to clot by leaving it undisturbed at room temperature (usually 15–30 minutes).
- 3. Centrifuge at $1000-2000 \times g$ for 10 minutes in a refrigerated centrifuge to separate the clotted material.
- 4. Immediately transfer the resulting supernatant (serum) into a clean polypropylene tube using a Pasteur pipette.
- 5. If the serum is not analyzed immediately, apportion the serum into 0.5 mL aliquots. Store and transport the aliquots at -20°C or lower.



Thaw reagents

- 1. Thaw all reagents except Ligase at room temperature.
- 2. Keep Ligase and thawed reagents on ice.

Reconstitute standard

1. Reconstitute one standard vial with Assay Dilution Buffer. See label on the vial for the reconstitution volume. Mix by gently inverting the vial five times. **Do not vortex**.

Note: Do mix by pipetting because the crystallized powder can be trapped in the pipette tip. If the powder does not go into solution after a few minutes, repeat the inversion.

2. Incubate for 15 minutes at room temperature.

Prepare working plate (on cold block 4°C)

Mix antibody-conjugates

The volumes provided in the following table are sufficient to run an entire 96-well plate using a 50 μ L reaction volume. The volumes can be scaled proportionally depending upon the number of assay wells being used and the reaction volume. If using 20 μ L reaction volumes, 2 μ L of antibody-conjugate mixture is required for each assay well. If using 50 μ L reaction volumes, 5 μ L of antibody-conjugate mixture is required for each assay well.

1. Add the following components to a 1.5-mL microcentrifuge tube, then mix by pipetting up and down.

Component	Volume	
Antibody-conjugate A	12 µL	
Antibody-conjugate B	12 µL	
Antibody-conjugate Dilution Buffer	696 µL	

2. Dispense $\ge 80 \ \mu$ L of antibody-conjugate mixture to each well in one column of the working plate.

Note: This step may not be needed if only running partial plates or not using a multi-channel pipette.

Dilute samples 10-fold

Sample dilution is performed directly in the working plate.

1. Add the volumes of sample and Assay Dilution Buffer in each assay well of the working plate.

Component	20 µL reaction	50 µL reaction
Assay Dilution Buffer	18 µL	45 µL
Unknown sample	2 μL	5 µL

2. Mix the contents of the assay wells by pipetting up and down.

Create standard curve

- 1. Add 80 μ L of Assay Dilution Buffer to each well in one column of the working plate that is designated for standards.
- 2. Mix reconstituted standard by gently inverting the vial five times. Transfer 20 μ L of the protein standard to the first well (S1), then mix.

Note: The total volume in protein standard wells is 100 μ L in order to minimize the CV for standard curve calculation.

3. Make serial dilutions of the standard by transferring 20 μL from well S1 to well S2. Continue serial dilution to well S7. The Background well (B) should only contain Assay Dilution Buffer. Mix wells thoroughly. Change pipette tips between dilution steps.



Note: The standard curve can be modified to extend the range. See "Guidelines for preparing and calculating standard curves" on page 8.

- 4. Seal the working plate with an adhesive seal using the plate seal hand tool.
- 5. Mix by striking the side of the plate against the palm of your hand three times. Rotate the plate and strike the other side of the plate against your palm three times.



- **6.** Centrifuge at $3,000 \times g$ for 1 minute to collect the liquid at the bottom of all wells.
- 7. Discard remaining unused reconstituted standard.

Run assay

Bind analyte (1 hour)

Keep the assay plate on a plate rack except during mixing or centrifugation steps.

1. Use a multichannel pipette to transfer the volume of antibody-conjugate mixture appropriate for your reaction volume from the working plate to all assay wells in the assay plate.

20 µL reaction	50 µL reaction	
2 µL	5 µL	

2. Transfer the volume of standards or diluted samples appropriate for your reaction volume from the working plate to the appropriate wells in the assay plate. Mix thoroughly by pipetting up and down several times.

20 µL reaction	50 µL reaction	
2 µL	5 µL	

- **3.** Seal the assay plate with an adhesive seal. Mix by striking the plate as previously described. Centrifuge at $3000 \times g$ for 1 minute.
- 4. Incubate the assay plate for 1 hour at room temperature, or if desired, overnight at 4°C.

Perform qPCR

1. Add 5 mL of Master Mix and 30 μ L of Ligase to a 15-mL conical tube. Mix thoroughly by pipetting up and down, then pour the qPCR reaction mixture into a reagent reservoir.

Note: These volumes are sufficient to run an entire 96-well plate using a 50 μ L reaction volume. The volumes can be scaled proportionally depending upon the number of assay wells being used and the reaction volume.

2. Add the volume of qPCR reaction mixture appropriate for your reaction volume to all assay wells. Mix by pipetting up and down. Avoid introducing bubbles in the wells.

20 µL reaction	50 µL reaction
16 µL	40 µL

- **3.** Seal the assay plate with an optical plate seal. Ensure there is complete adhesion using the plate seal hand tool.
- 4. Mix by striking the plate as previously described, then centrifuge at $3,000 \times g$ for 1 minute.



5. Input qPCR instrument settings.

Parameter	Settings for Applied Biosystems [™] instruments ^[1]		
Experiment type	Standard Curve or Quantitation - Standard Curve		
Reagents	TaqMan [™] reagents		
Reporter dye	FAM		
Quencher	NFQ-MGB ^[2]		
Passive reference	ROX		
Assign wells	Define all wells of the 96-well plate as Unknown		
Threshold	0.2		
Baseline	3–15		

For non-Applied Biosystems instruments, collect Ct values for each data point using the equivalent settings in the table.
 For instruments without this option, enter "None" or "Non-fluorescent".

6. Run the PCR plate using the protocol conditions appropriate for the block type.

		Tir			
Step	Temp (°C)	Standard Protocol	Fast Protocol ^[1]	Stage	
Ligation	25	20 min	20 min	Hold	
Ligase inactivation	95	2 min	2 min	Hold	
Denaturation	95	15 s	1 s		
Annealing/ extension	60	1 min	20 s	40 Cycles	

^[1] Use default settings for 7500, 7500 Fast, 7900HT, or non-Applied Biosystems instruments (e.g., 3 second denaturation step and 30 second annealing/extension step).

7. Import .eds, .sds., or .cvs files to the ProQuantum[™] software (available at apps.thermofisher.com/ apps/proquantum) to generate standard curves and determine sample concentrations.

Note: For non-Applied Biosystems instruments, see "Settings for non-AB instruments" on page 39 for details on handling .cvs files.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/ global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



Troubleshooting

Observation	Possible cause	Recommended action
No Ct values in data file	qPCR software was not set up properly.	Make sure that all 96 wells are designated as unknown.
		Make sure that the parameters including FAM, ROX passive was set.
		Make sure the camera collected is at the last cycle point.
No data collected error ProQuantum Software - error message that no data was collected	File was saved to a USB memory device directly from the instrument and not from the connected PC.	Go back to the computer and locate the file and save it from the computer and not directly from the instrument.
Poor standard curve (poor recovery)	Improper serial dilution.	Verify that amounts of Assay Dilution Buffer and recombinant protein is correct for each well.
		Verify that the range of dilutions are within the recommended range in the kit protocol.
	Contamination from well to well.	Make sure that the plates are sealed tightly so that no spillage happens during mixing and plate centrifugation.
		Make sure to change pipette tips in between wells or samples.
Poor standard curve (high CV)	Pipetting issue.	Verify that the pipettes are calibrated.
		Make sure to use low retention filter tips, which are important for small volume pipetting.
		Make sure that bubbles are minimized when pipetting up and down.
		Use best practices with pipetting, i.e., pipette liquid onto the side of the plate, visual examination.
		Use multi-channel pipettes where possible.
		Ensure that each pipette tip is tightly secured with the visually correct amount of liquid.
		Do not do reverse pipetting with small volumes.
		If small volume such as 2 μ L is a problem with consistency, try increasing the volume to 5 μ L.



Observation	Possible cause	Recommended action
Poor standard curve (high CV) (continued)	Improper mixing.	Verify that the plate is sealed properly using a hand tool.
		When mixing, make sure it is thoroughly done (i.e., pipette up and down 10 times or striking the plate hard enough so that liquid goes from one side of the well to the other) at all steps when introducing new components.
		Make sure that centrifugation of the plate occurs after the mixing step to ensure all reagents come to the bottom of the plate.
	Evaporation when using small volumes.	Minimize any setup time by using the working plate or another method so that small volumes are not exposed for long periods of time.
	Insufficient replicates.	Run samples in triplicate so that outliers are more easily identified.
Poor standard curve (high low end CV) High CV only at the low end of the curve but not the linear portion of the curve	The assay is at the limit of sensitivity.	Acceptable data is when the CV is less than 20%, so data cannot be reliable in this range.



Instrument settings

Settings for running ProQuantum[™] immunoassays on Applied Biosystems[™] qPCR instruments are provided in the following section.

On Applied Biosystems[™] instruments, create a Method with the parameters (instrument settings, desired reaction volume, and PCR conditions) appropriate for your specific instrument. This Method can be saved as a template, and reused on any future runs that share identical parameters.

Determine ramp rate (QuantStudio[™] instruments only)

When using a 50 µL reaction volume with a QuantStudio[™] instrument, determine the ramp rate to be used for defining the Method.

- 1. Go the Run screen and change the reaction volume to 50 µL.
- 2. An error message will display. Click OK.



OK

- 3. Click Start run.
- 4. A dialog box will appear and display the maximum ramp rate for each step for the volume. Copy the ramp rate values to use in defining the method.
- 5. Click No.

Start Ru	n Validation			×
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	Do you wa	nt to proceed?		
		<u>Y</u> es	No	

6. Edit the ramp rate when creating your Method with the appropriate values taken from the instrument.

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Experimer	nt Method			🖉 Action 🗸 🛛	_ë Save ∨
	Volume	Cover			
	50 µL	105.0 °C			
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	Step1	Step1	Stepz		
Legends:	Data Collection On	Data Collection Off	Pause On II Pause Off	Advanced Settings V VeriFlex	
					Next

Settings for QS 3 and 5 Systems

Settings for the QuantStudio[™] 3 System and QuantStudio[™] 5 System can be modified from the **New** experiment screen.



Set up experiment properties

- 1. Enter "ProQuantum" as the **Experiment name**.
- 2. Select your instrument type (QuantStudio[™] 3 System or QuantStudio[™] 5 System).
- 3. Select your block type (0.1 mL or 0.2 mL).
- 4. Ensure experiment type is set to **Standard curve**.
- 5. Ensure chemistry is set to TaqMan[™] Reagents
- 6. Select the appropriate run mode based on your block type (Fast for 0.1 mL, or Standard for 0.2 mL).
- 7. Click Next, and proceed to next step.

Properties Method Plate Re	un Results Export	
Experiment Properties		D ₈ Save v
Name ProQuantum	1)	Comments - optional
Barcode Barcode - optional		
User name Gate (1811) appear	(2)	
Instrument type QuantStudio" 5 Sys		
Block type 96-Well 0.1-mL Bloc		
Chemistry TaqMan® Reagents		
Run mode Fast	(5)	
Manage chemistry of	setails	
6		
		7



Assign targets and samples

- 1. Assign all wells as unknown by setting wells to U.
- 2. Ensure passive reference is set to ROX.
- 3. Save Method.



Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

- 1. Add a new Hold stage.
- 2. Set first Hold stage to 25°C for 20:00.
- 3. Modify second Hold stage to 95°C for 2:00.
- 4. Set Reaction volume per well to 20 µL or 50 µL depending upon the protocol to be performed.

Note: The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio[™] instruments only)" on page 17 for details.)

5. Click Next, and proceed to next step.



Settings for the QS 12K System

Settings for the QuantStudio[™] 12K Flex System can be modified from the **Create** screen.

QuantStu	ıdio™ 12k	(Flex	
Tools	Experiment	Run	Analyze
OpenArray® Sample Tracker Software	Open	ОрепАлтау®	ExpressionSuite Software
Instrument Console	Create	Q 96/384/Array Cards	TaqMan Genotyper Software
ReadiApp	Create From Template		DigitalSuite Software

Set up experiment properties

- 1. Enter "ProQuantum" as the Experiment name.
- 2. Select your block type (0.1 mL or 0.2 mL).
- 3. Ensure experiment type is set to **Standard curve**.
- 4. Ensure reagent type is set to TaqMan[™] Reagents
- 5. Select the appropriate instrument properties based on your block type (Fast for 0.1 mL, or Standard for 0.2 mL).
- 6. Click Define, and proceed to next step.

QuantStudio [™] 12	2K Flex Software v1.2.2			- Ø	23
File Edit Instrument	Analysis Tools Help				
🔣 New Experiment 🔹	📓 Open 🖬 Save 🔹 🖄 Gose 🦏 Import 🛛 🛷 Create Side	A Print Report			
Experiment Henry	Experiment: ProQuantum	Type: Standard Curve	Reagents: TaqMan	® Reagents	3
	How do you want to identify this experiment?				
Setup	Experiment Name: ProQuantum		Comments:		^
Experiment Properties	Barcooe: User Name:				-
Define 6	• Which block are you using to run the experiment?			2	
Assign	384-Well	Array Card	96-Well (0.2mL)	Fast 96-Well (0.1mL)	
Run Method	" What type of experiment do you want to set up?	3			
	✓ Standard Curve	Relative Standard Curve	Comparative Cr (ΔΔCr)	Melt Curve	
Run	High Resolution Melt	Genotyping	Presence/Absence		_
	"Which reagents do you want to use to detect the target	sequence?			
Analyss	✓ TaqMan⊗ Reagents	4 SYBR® Green Reagents	Other		
Event	[®] What properties do you want for the instrument run?	ÿ			
	Standard	✓ Fast)		
🚰 Home	2017-07-05 10×				

Define target

- 1. Enter "Unknown" as the Target name.
- 2. Ensure the **Reporter** is set to **FAM**.
- 3. Ensure the Quencher is set to NFQ-MGB.
- 4. Ensure the **Passive reference** is set to **ROX**.
- 5. Click Assign, and proceed to next step.

3

Elo Edit Instrument	Analysis Tools Help					- 0 X
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Setup	Targets New Save to Library Import from Library Delete	2 3)	Samples New SeveloLibrary Import from Library	Dwitte	
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Analysis	Biological Replicate Groups					
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	ROX					
G Home	1017-07-05 10 ×					

Assign wells

- 1. Assign all wells as unknown by setting Task to U.
- 2. Click Run method, and proceed to next step.



Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

- 1. Add a new Hold stage.
- 2. Set first Hold stage to 25°C for 20:00.
- 3. Modify second Hold stage to 95°C for 2:00.
- 4. Set Reaction volume per well to 20 µL or 50 µL depending upon the protocol to be performed.

Note: The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio[™] instruments only)" on page 17 for details.)

5. Save the Method.

File Edit Instrument	Analysis T	Tools Help 5	nport 🌆 Create Silde 📇	Print Report		
Experiment Henu	Experin	nent: ProQuantum-Unk	known	Type: Standard Curve	Reagents: TaqMan® Reagents	2
Setup	Run Met Reaction Graphics	hod Volume per Welk 50 µL	4			
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Run Method			3	Enable AutoDelta Starting Cycle:		
- Co-	100 °C		95.0 °C	95.0 °C		
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Settings for QS 6 and 7 Systems

Settings for the QuantStudio[™] 6 Flex System and QuantStudio[™] 7 Flex System can be modified from the **Experiment setup** screen.

QuantStudio	™ Real-Tim	e PCR Software
Set Up	Run	Analyze
Experiment Setup	Q QuickStart	Analyze Experiment
Template	Instrument Console	New Gene Expression Study
😨 ReadiApp		

Set up experiment properties

- 1. Enter "ProQuantum" as the **Experiment name**.
- Select your instrument type (QuantStudio[™] 6 Flex System, QuantStudio[™] 7 Flex System, or ViiA[™] 7 Real-Time PCR System).
- 3. Select your block type (0.1 mL or 0.2 mL).
- 4. Ensure experiment type is set to Standard curve.
- 5. Ensure reagent type is set to TaqMan[™] Reagents
- 6. Select the appropriate instrument properties based on your block type (Fast for 0.1 mL, or Standard for 0.2 mL).
- 7. Click **Define**, and proceed to next step.



Define target

- 1. Enter "Unknown" as the Target name.
- 2. Ensure the **Reporter** is set to **FAM**.
- 3. Ensure the Quencher is set to NFQ-MGB.
- 4. Ensure the **Passive reference** is set to **ROX**.
- 5. Click Assign, and proceed to next step.

3

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New Experiment •	🖉 Open 🚽 Save + 當 Close 🖷 Import • 📣	Create Side 📇 Print Report						
Experiment New	Experiment: ProQuantum		Type: Standard Curve			Reagents: TaqMan® Reagents		2
Setun	Targets New Save to Ubrary Import from Library Dekte	2	3		Samples New Save to Library Import from Library	Dwiete		
Experiment Properties	Target Name Unknown	Reporter Quent FAM V NFQ-M	cher Color GB 🗸	•	Sample Name Sample 1		Color	
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	4							
	Passive Reference							
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Assign wells

- 1. Assign all wells as unknown by setting Task to U.
- 2. Click Run method, and proceed to next step.



Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

- 1. Add a new Hold stage.
- 2. Set first Hold stage to 25°C for 20:00.
- 3. Modify second Hold stage to 95°C for 2:00.
- 4. Set Reaction volume per well to 20 µL or 50 µL depending upon the protocol to be performed.

Note: The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio[™] instruments only)" on page 17 for details.)

5. Save the Method.



Settings for the StepOnePlus System

Settings for the StepOnePlus[™] Real-Time PCR System can be modified from the **Advanced setup** screen.

New Experiment • 📓 Open 📓 Save • 🛍 Close 🕼 Send Experiment to Instrument.	🌄 Download Experiment from Instrument 🥔 Export + 💄 Print Report	
Set Up	Run	Analyze
Design Wizard	QuickStort	Analyze Experiment
Advanced Setup		
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Save current display as the default	Step 0ne & Step 0ne Plus	Agelied Biosystems Home Real-Time POR Decision Tree

Set up experiment properties

- 1. Enter "ProQuantum" as the **Experiment name**.
- 2. Select your instrument type (StepOnePlus[™] Instrument (96 Wells or 48 Wells)).
- 3. Ensure experiment type is set to **Standard curve**.
- 4. Ensure reagent type is set to **TaqMan[™] Reagents**
- 5. Select the appropriate instrument properties based on your block type (Fast for 0.1 mL, or Standard for 0.2 mL).
- 6. Click Plate setup, and proceed to next step.



Define targets and samples

- 1. Enter "Unknown" as the **Target name**.
- 2. Ensure the **Reporter** is set to **FAM**.
- 3. Ensure the Quencher is set to NFQ-MGB.
- 4. Click Assign targets and samples, and proceed to next step.

🔝 New Experiment + 📴 Open.	📓 Save 🔹 🖆 Glose 🔛 Send Experiment to Instrument 🕅 Do	wnload Experiment from Instrument 🛷 Export • 🏯 Print Report			
Experiment Menu «	Experiment: ProQuantum	Type: Standard Curve	Reagents: TaqMan® Reagents	START RUN 📡	2
Setup	Define Targets and Samples Assign Targets and	d Samples			
Experiment Properties	Instructions: Define the targets to quantify and the samples to tes Define Targets	at in the reaction plate.	Define Samples		
Plate Setup	Add New Target Add Saved Target Save Target Delete Targ		Add New Sample Add Saved Sample Sove Sample Delete Sample		
Run Method	Target Name Reporter	Quencher Color	Sample Name	Color	
Reaction Setup	Unknown FAM	VFQ-MGB	Sample 1		-
Materials List					
Run					
Analysis					
	Define Biological Replicate Groups				
	Instructions: For each biological replicate group in the reaction p	plate, click Add Biological Group, then define the biological group.			
	Biological Group Name	Color	Comments		_
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Assign targets and samples

- 1. Assign all wells as unknown by setting Task to U.
- 2. Ensure the **Passive reference** is set to **ROX**.
- 3. Click **Run method**, and proceed to next step.



Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

- 1. Add a new Hold stage.
- 2. Set first Hold stage to 25°C for 20:00.
- 3. Modify second Hold stage to 95°C for 2:00.
- 4. Set Reaction volume per well to 20 µL or 50 µL depending upon the protocol to be performed.
- 5. Save the Method.



Importing .csv files



Data from qPCR instruments using 48-well blocks need to be imported as .csv files.

The .csv files need to have Ct data values formatted in rows and columns as represented on a 48-well plate as shown in the following layout. Do not include any headers with the .csv file.

	A	В	С	D	E	F	G	Н
1	13.417485	25.835571	17.634604	19.591108	26.777365	22.05344	24.465328	26.280548
2	25.531294	26.223822	26.246462	25.491188	25.321566	25.222649	26.042915	26.52998
3	25.489466	26.33618	26.282022	25.27978	26.006775	26.03464	26.18903	26.124218
4	25.222649	25.284784	25.775894	26.486937	25.348553	26.050512	26.934269	26.678078
5	15.477636	17.540136	25.805576	19.979145	22.435043	25.72326	24.326393	25.418428
6	27.274868	25.869804	26.293247	27.218275	26.051727	25.395878	25.971722	25.08545

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Settings for the 7500 System

Settings for the 7500 Fast Real-Time PCR Instrument can be modified from the **Advanced setup** screen.



Set up experiment properties

- 1. Enter "ProQuantum" as the **Experiment name**.
- 2. Select your instrument type (7500 or 7500 Fast).
- 3. Ensure experiment type is set to Standard curve.
- 4. Ensure reagent type is set to TaqMan[™] Reagents
- 5. Select the appropriate instrument properties based on your block type (Fast for 0.1 mL, or Standard for 0.2 mL).
- 6. Click Plate setup, and proceed to next step.



Define targets and samples

- 1. Enter "Unknown" as the **Target name**.
- 2. Ensure the Reporter is set to FAM.
- 3. Ensure the Quencher is set to NFQ-MGB.
- 4. Click Assign targets and samples, and proceed to next step.

7500 Software v2.3				- Ó X
File Edit Instrument Analysis	Tools Help			
📧 New Experiment + 🥁 Open.	🚽 Save 🔹 🖆 Close 🌆 Export 🔹 📇 Print Report			
Experiment Menu «	Experiment: ProQuantum	Type: Standard Curve	Reagents: TaqMan@ Reagents	START RUN ()
Setup	Define Targets and Samples Assign Targets and Sa	mples		
Experiment Properties	Instructions: Define the targets to quantify and the samples to test in the Define Targets	reaction plate.	Define Samples	
Plate Setup	Add New Target Add Saved Target Save Target Delete Target	y y	Add New Sample Add Saved Sample Save Sample Delete Sample	
Run Method	Target Name Reporter	Quencher Color	Sample Name	Color
Reaction Setup	EAM EAM	VIFQ-MGB V	Sample 1	
Materials List				
Run				
Analysis				
	Define Biological Replicate Groups			
	Instructions: For each biological replicate group in the reaction plate, clin	ck Add Biological Group, then define the biological group.		
	Add Biological Group Delete Biological Group			
	Biological Group Name	Color	Comments	
«				Assign Targets and Samples
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Assign targets and samples

- 1. Assign all wells as unknown by setting Task to U.
- 2. Ensure the **Passive reference** is set to **ROX**.
- 3. Click **Run method**, and proceed to next step.

7500 Software v2.3			- @ X							
File Edit Instrument Analysis	Tools Help									
🔝 New Experiment 🔹 📴 Open.	. 🛃 Save 🕶 🖆 Close 🏼 🌆 Export 🔹 🖓 Print Report									
Experiment Menu «	Experiment: ProQuantum	Type: Standard Curve Reagents: TaqMan® Reagents	START RUN 😥 🕐							
Setup	Define Targets and Samples Assign Targets and Samples									
Experiment Properties	To set up standards: Click "Deline and Set Up Standards." Instructions: Set up uninowns: Seted velta, assign target(s), a seted "U" (Uninown) as the task for each target assignment, then assign a sample. To set up ungeblex controls Seted setup(s), samples (set), then seted "W" (Negable Control) as the task for each target assignment.									
Plate Setup	Assign target(s) to the selected wells	View Plate Layout View Well Table								
Run Method - 3	Assign Target Task Quantity	Select Wells With: [-Select filem -]								
Reaction Setup	Urknown	Show in Wells E View Legend								
		1 2 3 4 5 6 7 8 9 10	11 12							
Materials List		A Sample 1 Utroan 1 Utroan 1 Utroan 1 Utroan	Sample 1 Sample 1							
Analysis	ک الندط الله الندو الله الندان الله الله الله الله الله الله الله ال	B Sangle 1 S	Sample 1 Sample 1							
	Assign sample(s) to the selected wells.	C Scole 1 Scol	Sample 1 Sample 1							
	Sample 1	D Sangle 1 S	Sample 1 Sample 1							
	Assim sample(s) of selected well(s) to biological group	E Sangh 1 Sang	Sample 1 Utinown Utinown							
	Assign Biological Group	F Escript Sample	Sample 1 Urknown							
		0 Samplet S	Sample 1 Sample 1							
	Select the dye to use as the passive reference.	H Samplet S	Sample 1 Sample 1							
	Rox 2	Wells: 🚺 96 Unknown 🔂 0 Standard 🔝 0 Negative Control	0 Empty							
Home 🔚 Untitled ×	р									

Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

- 1. Add a new Hold stage.
- 2. Set first Hold stage to 25°C for 20:00.
- 3. Modify second Hold stage to 95°C for 2:00.
- 4. Set Reaction volume per well to 20 µL or 50 µL depending upon the protocol to be performed.
- 5. Save the Method.



Settings for non-AB instruments

If you are using a qPCR instrument from another manufacturer, collect Ct values for each data point using the equivalent settings described in the following table.

Parameter	Settings for Applied Biosystems [™] instruments					
Experiment type	Standard Curve or Quantitation - Standard Curve					
Reagents	TaqMan [™] reagents					
Reporter dye	FAM					
Quencher	NFQ-MGB ^[1]					
Passive reference	ROX					
Assign wells	Define all wells of the 96-well plate as Unknown					
Threshold	0.2					
Baseline	3–15					

^[1] For instruments without this option, enter "None" or "Non-fluorescent".

Importing .csv files

Data from qPCR instruments produced by other manufacturers need to be imported as .csv files.

	CSV Import												×	
E	Before clicking Next: Make sure the well data in the file is arranged in rows and columns like a plate.													
		A	В	С	D	E	F	G	Н	I	J	К	L	
	1	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	
	2	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	
	3	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	
	4	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	
	5	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	
	6	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	
	7	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	
	8	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	
											-			
	Cancel Nex										Next			

The .csv files from non-Applied Biosystems instruments need to have Ct data values formatted in rows and columns as represented on a 96-well plate as shown in the following layout. Do not include any headers with the .csv file.

	A	В	С	D	E	F	G	Н	1	J	K	L
1	13.417485	25.835571	17.634604	19.591108	26.777365	22.05344	24.465328	26.280548	25.935783	27.044222	26.877268	26.834337
2	25.531294	26.223822	26.246462	25.491188	25.321566	25.222649	26.042915	26.52998	25.951874	29.698883	25.984228	25.489466
3	25.489466	26.33618	26.282022	25.27978	26.006775	26.03464	26.18903	26.124218	26.179691	25.951874	26.76191	26.373287
4	25.222649	25.284784	25.775894	26.486937	25.348553	26.050512	26.934269	26.678078	26.233883	29.54913	26.552887	26.263578
5	15.477636	17.540136	25.805576	19.979145	22.435043	25.72326	24.326393	25.418428	26.436768	27.017548	26.827032	26.700144
6	27.274868	25.869804	26.293247	27.218275	26.051727	25.395878	25.971722	25.08545	26.522614	3.4741602	26.413948	28.00413
7	25.90196	25.222649	26.21743	26.222464	25.189606	26.246462	26.463821	25.861534	25.971722	26.372057	25.813805	25.760149
8	25.333206	27.274868	25.303984	26.284954	27.218275	25.53206	25.848406	28.091894	25.992739	25.578075	27.65518	26.68899



