qPCR Validation Test 1

Version: 1.0
Release: April 2025

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OVERVIEW

The qPCR Validation Test 1 (DX-0101-01) is designed to introduce users to the qPCR method, practice accurate micropipetting, and ensure proper operation of their qPCR instrument. One kit provides enough materials for 16 reactions.

PROTOCOL AT A GLANCE

The test consists of the following steps:

Step		Time
1	qPCR setup	10 min
2	qPCR	1 hour and 40 min
	*Optional stopping point: once you complete the qPCR run and save the file, you can proceed with data analysis whenever is convenient to you*	
3	Result analysis	10 min

KIT COMPONENTS

SUPPLIED IN KIT

Reagents and Supplies	DX-0101-01	Storage
qPCR Enzyme 1	1 tube	Freezer
qPCR Validation Probe 1	1 tube	Freezer
qPCR Validation Positive Control 1	1 tube	Freezer
Water	1 tube	Freezer
PCR tubes	1 bag	Room temp.

SUPPLIED BY USER

Equipment

All items are available at https://dx.minipcr.com.
If you're starting your lab, we recommend the qPCR Lab (QP-2510-50).

Item	Quantity	Recommended product	Cat. Number
Real time thermal cycler	1	q16 thermal cycler	QP-1100-01
Micropipettes	1 each	1-10 µl H-style	QP-1001-05
		2-20 µl H-style	QP-1001-01
		100-1000 µl H-style	QP-1002-02
		4 µl fixed volume minipette	QP-1003-01
		20 µl fixed volume minipette	QP-1003-03
Microcentrifuge tube rack	1	Microcentrifuge tube rack, 60-well	CM-1003-10
PCR tube rack	2	0.1 ml PCR tubes rack - 96 wells	CM-1003-11
Microcentrifuge	1	Gyro™ Microcentrifuge, fixed speed	QP-1800-01

Consumables

All consumables are one-time use.

Item	Quantity	Recommended product	Cat. Number
Micropipette filter tips	2 boxes each (sufficient for processing up to 190 samples)	1-10 µl	4AA75
		2-20 µl	4AA76
		20-200 µl	4AA77
		100-1000 µl	4AA78
Microcentrifuge tubes	1 tube per run	Microcentrifuge tubes 1.5 ml	6AA02

Other laboratory supplies

- Disposable laboratory gloves
- Protective eyewear
- Lab coat
- 2 Bottles or sprayers with bleach solution (prepare freshly by mixing 1 part household bleach +1 9 parts water)
- 2 Permanent markers
- 3 containers with lid to dispose of used tubes and tips
- Tissue paper

BEFORE YOU START

LABORATORY GUIDELINES

qPCR is an extremely sensitive technique that can detect minute amounts of RNA or DNA. Always follow the practices outlined below to minimize the risk of contamination.

	Reagents area	Sampling area	qPCR area
Purpose	Preparing master mix	Transferring water or the positive control to master mix	Running the qPCR test Data analysis
Precautions	No positive control or completed PCR reactions should be brought to this area	Material exposed to the Sampling area should not be returned to the Reagents area	Do not open the tubes after the qPCR step.
Materials	*Reagents* qPCR Enzyme 1 qPCR Validation Probe 1 *Equipment* 20-200 µl and 100-1000 µl micropipettes 20 µl fixed volume minipette Marker Tube racks Trash bin Bottle with bleach solution *Consumables* Tissue paper Gloves 20 µl, 200 µl, and 1000 µl filter tips PCR and 1.5 ml tubes	*Reagents* qPCR Validation Positive Control 1 Water *Equipment* Microcentrifuge 1-10 µl micropipette 4 µl fixed volume minipette Marker Tube racks Trash bin Bottle with bleach solution Wash bottle with distilled water *Consumables* Tissue paper Gloves 10 µl and 20 µl filter tips Tissue paper	*Reagents* No reagents *Equipment* q16 thermal cycler USB drive USB extension cable Windows or Mac computer Trash bin *Consumables* A few empty PCR tubes (they can be reused)

BEST PRACTICES

- Clean your hands and wear a clean lab coat before running a test
- Maintain clean work areas. Spray freshly prepared bleach solution (1 part bleach + 19 parts water) on work surfaces before and after every use.
- Always use filter tips. Do not use the tips included with the minipettes as those don't have filters.
- Wear a clean lab coat when preparing the reagents.
- Discard materials such as tips, tubes, and gloves in a container that can be closed. Empty it often and clean it with a bleach solution.
- Keep all tubes closed except for the one that you are actively using.

Failure to follow good laboratory practices can result in contamination that will invalidate results. A contaminated laboratory is difficult to clean up.

CONTROLS

Positive and negative controls are mandatory.

To ensure accurate results and proper interpretation, each run **must include** both a negative and a positive control. Omitting these controls will hinder the software''s ability to automatically analyze the data.

Negative control (mandatory)

The negative control will always be assigned to position 1 in the q16 thermal cycler. Follow manufacturer recommendations for other qPCR thermal cyclers.
Amplification in the negative control indicates contamination and invalidates the results of the test batch.

Positive control (mandatory)

The positive control will always be assigned to position 2 in the q16 thermal cycler. Follow manufacturer recommendations for other qPCR thermal cyclers.

Handle with care to avoid contaminating other tubes, equipment, and surfaces. Contamination with the positive control will result in false positive tests.

Amplification in the positive control indicates the test is working as expected but does preclude the possibility of contamination.

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q16 THERMAL CYCLER

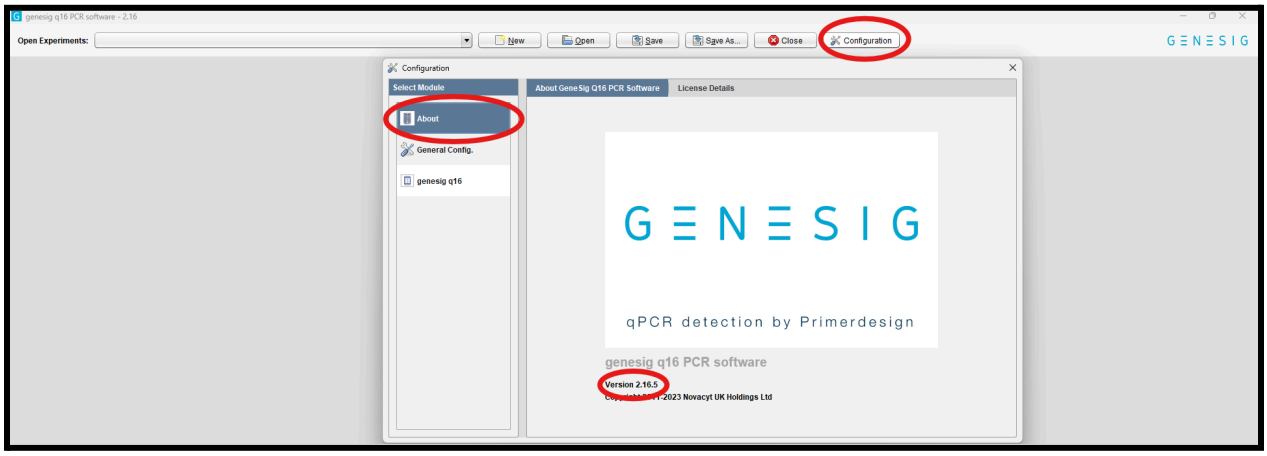
Read the q16 user's guide before proceeding. This is a quick guideline.

q16 software version 2.16.5 & q16 user's guide

Install the software version 2.16.5

- **The software is available on our website (dx.minipcr.com)**
- **The USB drive provided with your q16 thermal cycler contains software for Windows and MacOS operating systems**. Please open the software file matching your chosen operating system.
    - *Windows:* Double-click on the Windows installer and follow the on-screen instructions to install your genesig® q16 software on Windows.
    - *MacOS:* Double-click on the disk image and follow the on-screen instructions to install your genesig®q16 software on your Mac computer.

IMPORTANT: make sure to install version 2.16.5 of the software. Previous versions are not compatible with this kit. You can verify the installed version by clicking under Configuration/About.



PROCEDURE

Please read these instructions completely before continuing.

SETUP

We recommend preparing one negative control, one positive control, and six sample replicates, to evaluate accurate micropipetting technique.

Suggested sample labeling

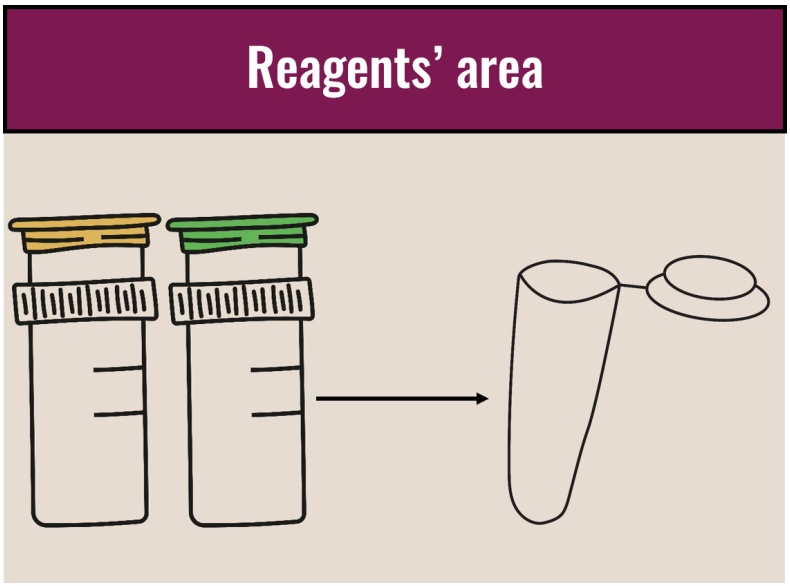
PCR Tube ID	Default name on software
1	Negative control
2	Positive control
3	Sample 1
4	Sample 2
5	Sample 3
6	Sample 4
7	Sample 5
8	Sample 6

Tubes needed per run

Reactions	Tubes Needed
6 Tests 1 Negative control 1 Positive control	8 PCR Tubes

qPCR Validation Test 1

STEP 1. qPCR SETUP



In this step, you will prepare the master mix in the reagents area. You will need the qPCR Enzyme 1, the qPCR Validation Probe 1, and the PCR tubes.

1. Wipe all surfaces with freshly prepared cleaning solution.
2. Thaw the provided reagents (qPCR Enzyme 1 and the qPCR Validation Probe 1) at room temperature.
3. Prepare the master mix by following the instructions in the table below.
   -These calculations account for loss of volume during pipetting. You may have a small amount of liquid left in the tube.
   -The total number of reactions is the sum of the number of tests, the negative, and the positive controls.

Reagent	Volume for 1 reaction (µl)	Volume for 8 reactions (µl)
qPCR Enzyme 1	11	88
qPCR Validation Probe 1	11	88
Total volume	**22**	**176**

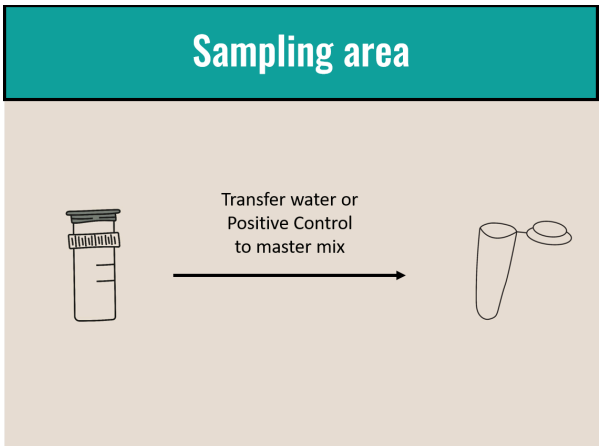
4. Add the reagents to a 1.5 ml microcentrifuge tube. Gently mix up and down until the solution is well mixed.
5. Using the dedicated **20 µl fixed volume minipette** with a **2-20 µl filter tip** to add 20 µl of the PCR mix to each PCR tube. There's no need to change tips at this time.
6. Close the tubes.
7. Label tubes with the proper ID on the top.

Test	PCR Tube ID
Negative control	1
Positive control	2
Sample 1	3
Sample 2	4
Sample 3	5
Sample 4	6
Sample 5	7
Sample 6	8

8. Put the qPCR Enzyme 1 and the qPCR Validation Probe 1 back in the freezer.
9. Move the tubes to the sampling area.

qPCR Validation Test 1

STEP 2. qPCR



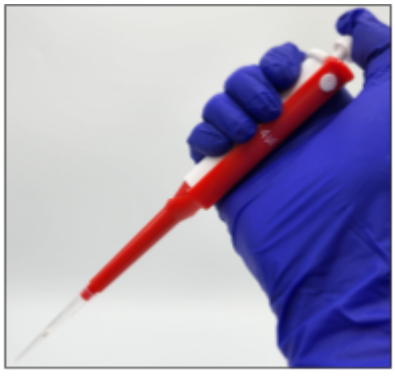
In this step, you will add the water or the qPCR Validation Positive Control 1 to the master mix and run the qPCR in the q16 thermal cycler.

Transfer the template to PCR mix

Open only one tube at a time to avoid contamination

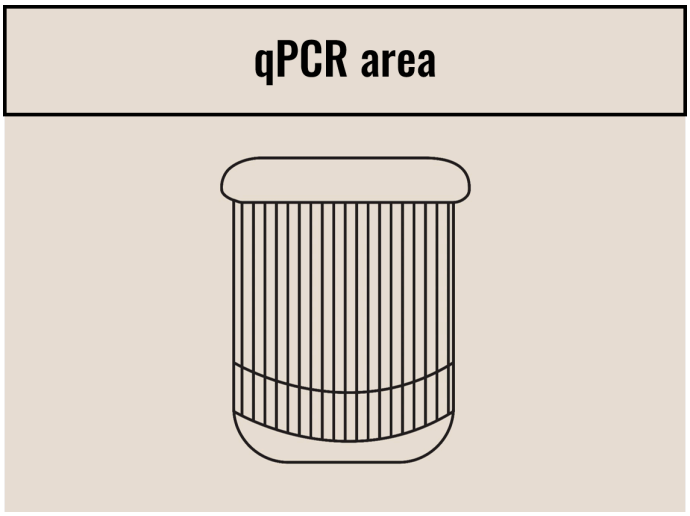
1. Thaw the water and the qPCR Validation Positive Control 1. Once thawed water can be stored at room temperature.
2. Process the negative control. Transfer 2 µl of water to the negative control PCR tube (tube 1) using the dedicated **H10 pipette** with a **1-10 µl filter tip**. Close both tubes tightly. Discard the tip.
3. Proceed with the tests. Transfer 2 µl from the qPCR Validation Positive Control 1 to the PCR tubes containing the master mix (tubes 3-8). Close both tubes tightly. Discard the tip.
4. Using the dedicated **4 µl fixed volume minipette** and a **2-20 µl filter tip**, transfer 4 µl of qPCR Validation Positive Control 1 to the tube labeled "2". Close both tubes.
5. Store the qPCR Validation positive Control 1 in the freezer.
6. **Check that all the PCR tubes containing the master mix are closed tightly.**
7. **Briefly spin down the microcentrifuge to remove bubbles**. Bubbles interfere with optical readings. Eliminating bubbles ensures accurate interpretation of the results.

Example of a 1-10 µl micropipette set to 2 µl


Example of 4 µl minipette with a 20 µl filter tip

qPCR Validation Test 1

Start the qPCR

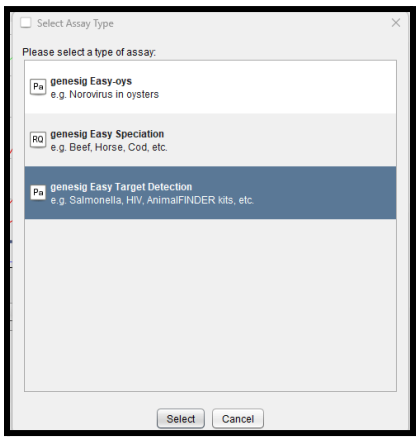


During this step you will run a qPCR using the q16 thermal cycler.

Please, read the genesig® q16 Instruction Manual before operating the q16 thermal cycler.

1. Connect the q16 thermal cycler to the power supply, then plug the power supply into an electrical outlet. The machine will make an audible announcement and the LED will display a blue static light to signal that the machine is on. There is no on/off switch. There is no on/off switch.
2. Connect also the USB adaptor cable to the bottom of the q16 thermal cycler.
3. Connect the USB Flash Drive to your computer.
4. Open the q16 app on your computer.
5. Select “New”.

6. Select “genesig Easy Target Detection”.

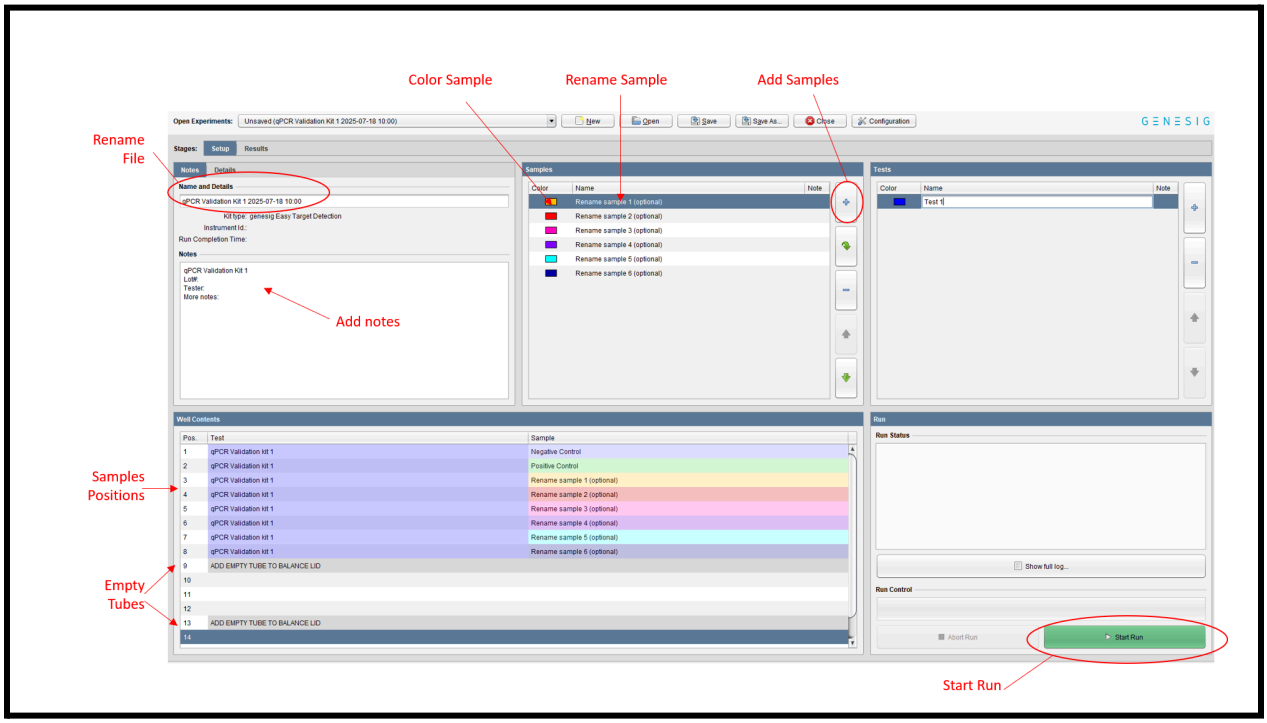


7. Rename the Experiment as you prefer.
8. Under Samples select “+” and add the number of samples based on your run.

Notes:
-The negative and positive control will be automatically assigned to position 1 and 2, respectively.
-The software will highlight where to add “empty” tubes, to make sure the lid will properly close.

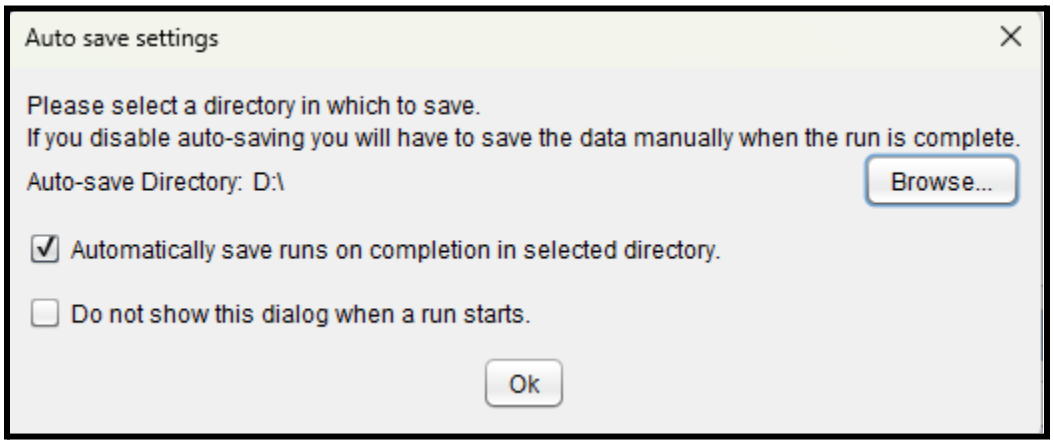
Optional: rename samples and change color by selecting the sample and typing in the box and/or selecting the relative box color.

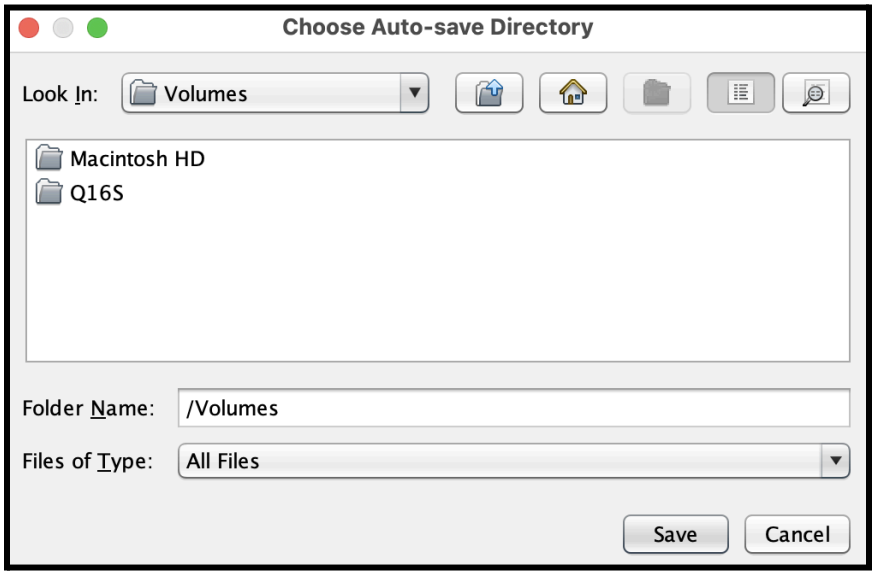
9. Click on “Start Run”. Note that the q16 thermal cycler will not yet start the program.



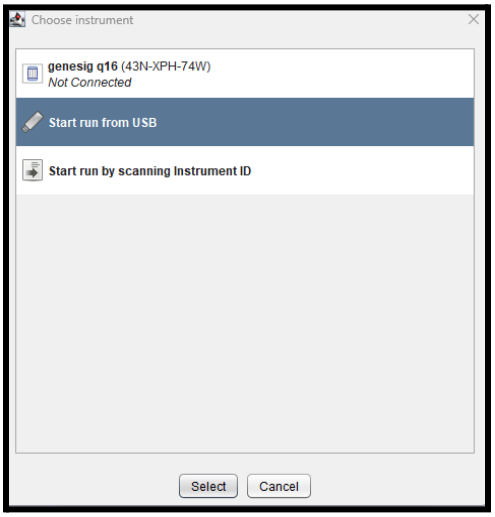
10. Select the USB drive as the directory for saving the data and click on “Ok”.

On a Mac, navigate to Volumes and select the name of the USB Drive.

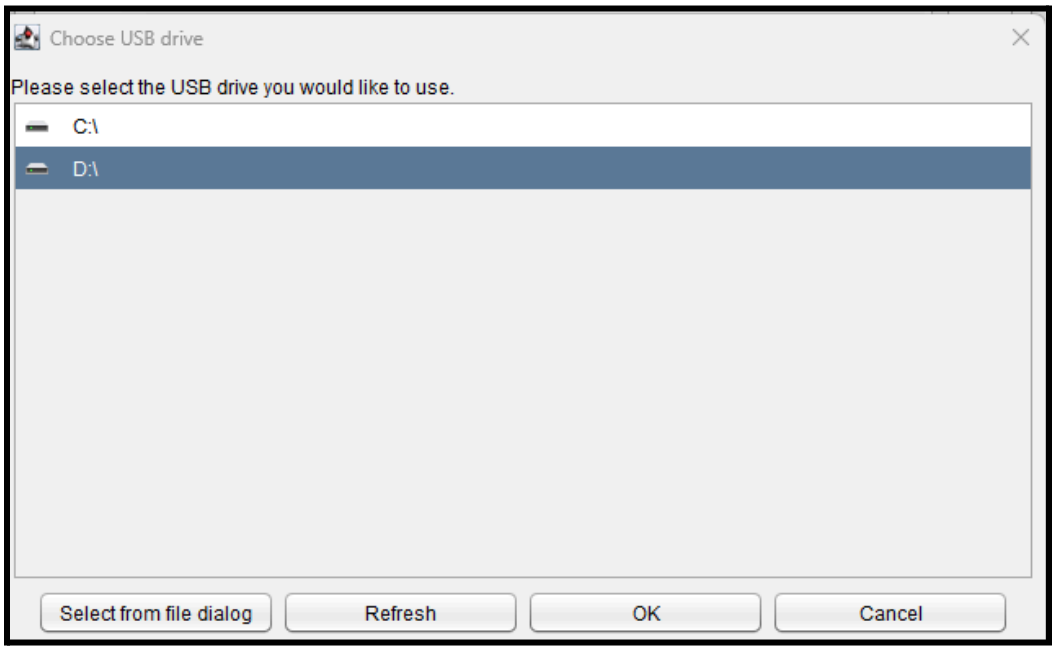




11. Select “Start run from USB” and click on “Select”.

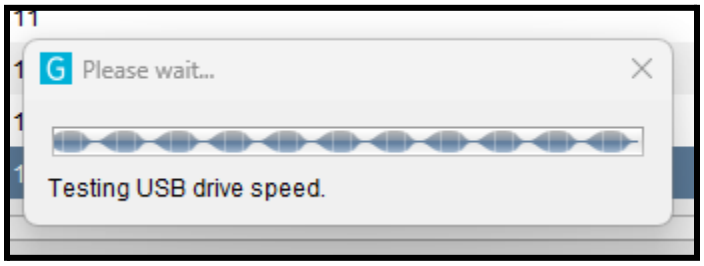


12. Select the USB drive and click “OK”.



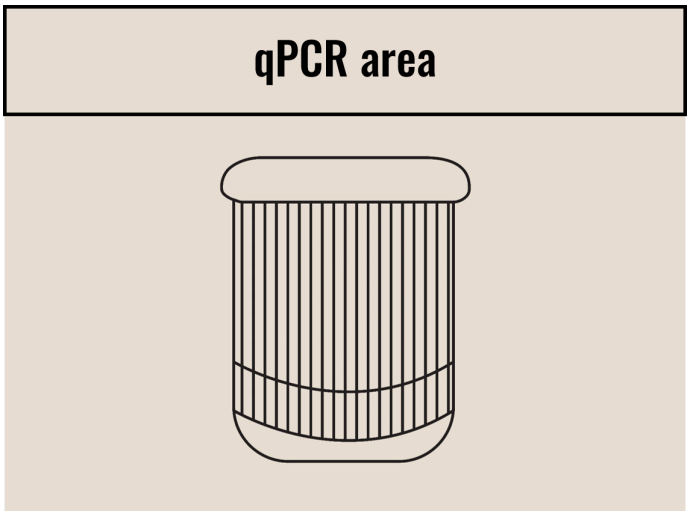
13. The software will test the USB drive. Once the test is correctly completed, remove the USB Flash Drive from your computer.

    Note: If the software detected the USB as too slow to load the program, try again repeating steps 9-13.



14. **To open the q16, push the lid down and twist a quarter turn anti-clockwise, then lift. Load the samples, making sure to add empty tubes (positions 9 and 13), as indicated by the software. Close the lid.**
15. **Plug the USB Flash Drive into the USB connector cable.**
16. When you start the run, the machine will make an audible announcement and the LED will turn green. The LED's color will change throughout the run, which takes approximately 1 hour and 40 minutes. A circling rainbow light pattern indicates that the program is completed.
17. Once the

STEP 3. RESULT ANALYSIS



In this step you will analyze the results.

The analysis requires checking both the automated result table as well as the amplification curves.

1. Open the file you would like to analyze.
2. Click on the “Results” tab.
3. Check the top “Summary” panel, be aware of possible warning messages.
4. Check the “Notes” panel for possible warning messages.
5. Click on “Details” close to the top “Summary” panel. Curves should appear.
6. Click on “Details” above the table close to the “Sample results” panel. The table should expand and show Cq values.
7. Negative control is labeled as “PASS” on the table.
8. Positive control is labeled as “PASS” on the table.
9. Check the results assigned by the software to each sample (see Result Interpretation and the Troubleshooting sections below).
10. Select a sample name from the table to highlight its amplification curve on the graph.
11. Manually examine the amplification plot for both the Test and Internal Control channels for each sample. Verify the presence of clear sigmoid curves, indicating successful amplification.
    a. Lack of a clear sigmoid curve indicates sub-optimal amplification and the run should be considered inconclusive.
    a. The samples and the positive control (green) curves should have a similar shape.
    b. Overlapping of the curves and similar Cq in the 5 sample replicates (Cq difference < 0.5) indicates accurate pipetting.

c. Expected Cq is approximately 15-20 for the Internal control and 15-20 for the Test.

12. Export the results for future record as .csv or .pdf (optional).

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Result Interpretation

Sample	Result	Curve Shape	Interpretation
Negative control	PASS	No amplification or curve showing up after Cq 36	Result as expected
Negative control	FAILED	Amplification and Cq < 36	Contamination. Tests should be considered invalid and they will be labeled as " TEST CONTAMINATED". Deeply clean the lab and repeat all the tests.
Positive control	PASS	Clear sigmoid curve for Test	Result as expected
Positive control	FAILED	No amplification or late amplification	-Inaccurate pipetting when adding the positive control -Issues with the master mix preparation -Possible issues with the q16 thermal cycler. Contact dx@minipcr.com If the positive control is detected as FAILED, the results will be labeled as "TEST FAILED".
Sample	POSITIVE	Clear sigmoid curve for both Test and Internal Control. Cq is similar to positive control.	
Sample	SAMPLE PREPARATION FAILED	No amplification or curve showing up after Cq 36	-Inaccurate pipetting when adding the positive control -Issues with the master mix preparation -Possible issues with the q16 thermal cycler. Contact dx@minipcr.com

EXPECTED RESULTS

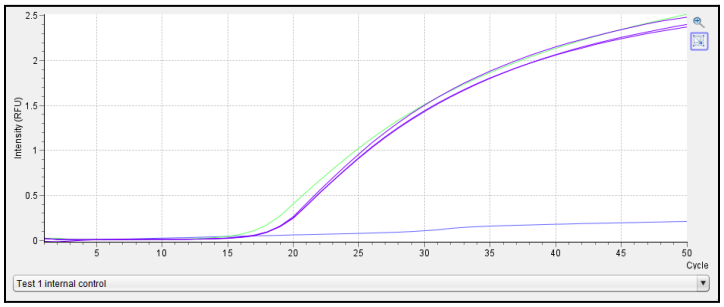
The test result must be interpreted using the result shown by the software (“Positive”, “Negative”, “Sample preparation failed”, “Positive result, poor quality sample”, or “Test failed”) and confirmed using the amplification graphs and the Cq value.

Results Table

#	Test	Sample	Status	Copy Number	Cq (Test)	Cq (I.C.)
1	Test 1	Negative Control	PASS	n/a	n/a	n/a
2	Test 1	Positive Control	PASS	n/a	18.55	16.50
3	Test 1	Sample 1	POSITIVE	1,269,029	19.21	17.70
4	Test 1	Sample 2	POSITIVE	1,248,157	19.23	17.74
5	Test 1	Sample 3	POSITIVE	1,330,922	19.14	17.64

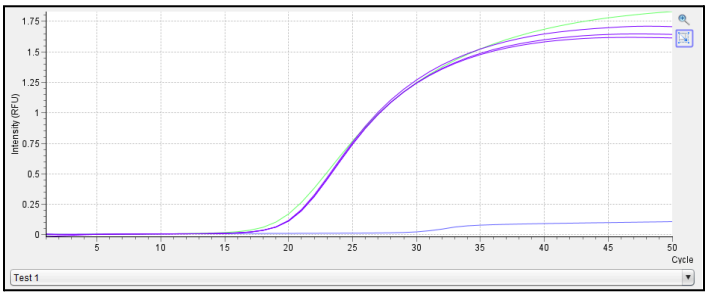
Internal control channel

- The *negative control* (light blue, flat line) should show a clear sigmoid curve.
- The samples (purple) and the positive control (green) curves should show a clear sigmoid curve.
- Overlapping of the samples (purple) curves and similar Cq (Cq difference < 0.5) indicates accurate pipetting.
- Expected Cq is approximately 15-20. The positive control (green) is expected to have earlier Cq than the samples.



Test channel

- The *negative control* (light blue, flat line) should show a clear sigmoid curve.
- The samples (purple) and the positive control (green) curves should show a clear sigmoid curve.
- Overlapping of the samples (purple) curves and similar Cq (Cq difference < 0.5) indicates accurate pipetting.
- Expected Cq is approximately 15-20. The positive control (green) is expected to have earlier Cq than the samples.



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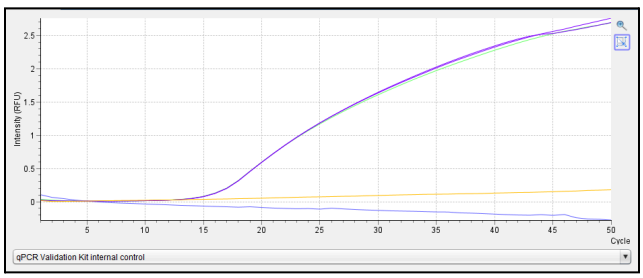
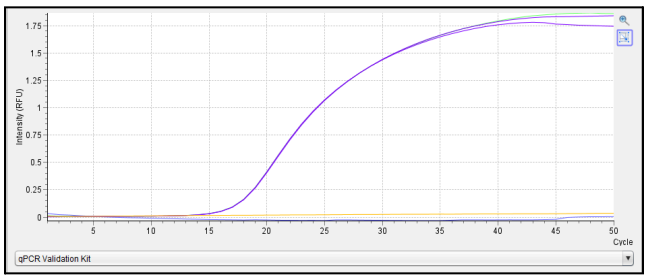
TROUBLESHOOTING

Example of pipetting error

Sample 3 (yellow) failed to amplify in both channels, indicating the template was likely not added. However, the successful amplification of the positive control (green) and samples 1 and 2 (purple), along with the expected flat line in the negative control (light blue), confirms the master mix and instrument are functioning correctly





#	Test	Sample	Status	Copy Number	Cq (Test)	Cq (I.C.)
1	qPCR Validation Kit	Negative Control	PASS	n/a	n/a	n/a
2	qPCR Validation Kit	Positive Control	PASS	n/a	16.56	15.14
3	qPCR Validation Kit	Sample 1	POSITIVE	1,969,638	16.58	15.19
4	qPCR Validation Kit	Sample 2	POSITIVE	1,912,595	16.62	15.20
5	qPCR Validation Kit	Sample 3	SAMPLE PREPARATION FAILED	n/a	n/a	n/a

APPENDIX

TUBE TYPES

Tube type	Picture	Description
Screw-cap reagent tube		Reagents for DNA and RNA extraction and RT-qPCR.
1.5 ml microcentrifuge tubes		Used to prepare the master mix. Not included in the kit. Available at dx.minipcr.com
PCR tubes		Used for the lysis and PCR steps. Included in the kit.

MICROPIPETTING

Good micropipetting skills are essential to run the test successfully. Practice with water or colored liquids before using the reagents in this kit.

Micropipetting video tutorial

We recommend dedicated micropipettes and minipettes per area:
-Reagents area: one 100-1000 µl micropipette, one 20-200 µl micropipette, and one 20 µl fixed volume pipette.
-Sampling area: one 1-10 µl micropipette and one 4 µl fixed volume minipette.

To reduce the contamination risk:

- Dedicate specific micropipettes and filter tip boxes to each area
- Always use filter tips
- Use only the dedicated minipette for transferring the RT-PCR master mix and the positive control. In case of contamination, dispose of the minipette and use a new one.

Always select the right micropipette and tip based on the volume that you need to transfer according to the tables in the following page.

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Volume range	Micropipette	Tips
1-10 µl	 H10	
2-20 µl	 H20	
20-200 µl	 H200	
100-1000 µl	 H1000	

Volume	Minipette	Tips
4 µl		
20 µl		

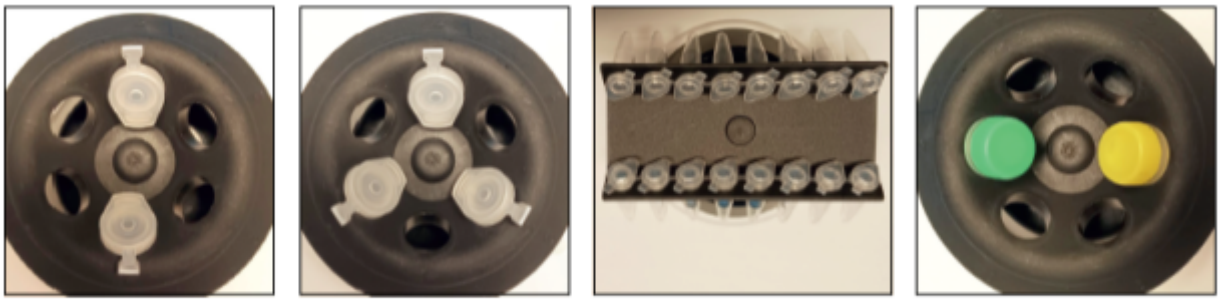
LIQUID HANDLING

Make sure that all liquid is collected at the bottom of the tubes before opening them.

Spin down the PCR tubes before the qPCR step. Bubbles can interfere with the qPCR process.

Follow these steps to use the microcentrifuge:

1. Ensure that the contents of the tubes are fully defrosted.
2. Place the tubes in the rotor, making sure they are balanced as shown in the pictures. The tubes should have approximately the same amount of liquid in them. If needed, prepare a tube with water to balance the rotor. Refer to the manufacturer's instructions for details.
3. Spin for 5 seconds at 10,000 RPM.



Examples of balanced microcentrifuges. Tubes match in size and contain the same volumes, making their weights comparable

SCIENTIFIC BASIS OF THE ASSAY

How to read and interpret qPCR data

A clear sigmoid curve in qPCR is crucial as it demonstrates successful and reliable amplification of the target DNA. This characteristic S-shaped curve signifies the exponential phase of PCR, where the amount of amplified product doubles with each cycle. The curve's plateau indicates the reaction has reached its endpoint, where amplification has ceased.

The cycle threshold (Ct/Cq) value is the cycle number at which the fluorescence signal crosses a defined threshold, indicating detectable amplification. It is inversely proportional to the initial amount of target DNA; a lower Cq indicates a higher starting concentration. Cq values exceeding 36 are generally considered unreliable because at such high Cq values, the signal is often very close to background noise, making it difficult to distinguish true amplification from random fluctuations. Additionally, potential inhibitors or degradation of reagents become more impactful in later cycles, further compromising the reliability of the results.

Consistent pipetting technique is vital for reliable results; technical replicates should ideally not vary by more than 0.5 cycles.