PowerQuant® System

Instructions for Use of Product PQ5002 and PQ5008.

Quick Protocol

This document is a quick protocol for experienced users to quantify DNA samples using the PowerQuant® System. For complete protocol information and troubleshooting tips, see the *PowerQuant® System Technical Manual* #TMD047, which is available online at: **www.promega.com/protocols/**

Serial Dilution of the PowerQuant® Male gDNA Standard

1. Ensure that the PowerQuant® Male gDNA Standard was stored at 4°C overnight before first use. Vortex the PowerQuant® Male gDNA Standard three times at high speed for 10 seconds each time.
2. Label three tubes with the following concentrations: 2ng/µl, 0.08ng/µl and 0.0032ng/µl.
3. Dilute the PowerQuant® Male gDNA Standard as indicated below. Vortex each dilution for 10 seconds prior to removing an aliquot for the next dilution. Change pipette tips between dilutions.

DNA Concentration	Volume of PowerQuant® Male gDNA Standard	Volume of PowerQuant® Dilution Buffer
50ng/µl	Use undiluted PowerQuant® Male gDNA Standard	0µl
2ng/µl	4µl of undiluted PowerQuant® Male gDNA Standard	96µl
0.08ng/µl	4µl of 2ng/µl dilution	96µl
0.0032ng/µl	4µl of 0.08ng/µl dilution	96µl

PowerQuant® Reaction Setup

1. Thaw the PowerQuant® 2X Master Mix, PowerQuant® 20X Primer/Probe/IPC Mix and Water, Amplification Grade, completely at room temperature.
2. Vortex the PowerQuant® 2X Master Mix and PowerQuant® 20X Primer/Probe/IPC Mix for 10 seconds to mix. Do not centrifuge after mixing.
3. Determine the number of reactions to be set up, including the no-template control reactions. Increase this number by 10–15%.
4. Prepare sufficient reaction mix by combining the volumes of Water, Amplification Grade, PowerQuant® 2X Master Mix and PowerQuant® 20X Primer/Probe/IPC Mix calculated below.

Component	Volume per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	7µl	×		=	
PowerQuant® 2X Master Mix	10µl	×		=	
PowerQuant® 20X Primer/Probe/IPC Mix	1µl	×		=	
Total volume	**18µl**				

5. Vortex for 10 seconds to mix. Do not centrifuge after mixing.
6. Add 18µl of reaction mix to the reaction wells of the MicroAmp® Optical 96-Well Reaction Plate.
7. Add 2µl of the PowerQuant® Male gDNA Standards prepared above or unknown DNA sample to the appropriate wells.
8. Add 2µl of TE^{-4} buffer (pH 8.0) or Water, Amplification Grade, to the no-template control reactions.
9. Seal the plate with MicroAmp® Optical Adhesive Film.
10. Centrifuge the plate briefly.

Starting a Thermal Cycling Run

Prior to thermal cycling, create a run template (.edt file) to store the dye information, target names and tasks; well locations and DNA concentrations for the DNA standards; run method and analysis settings. Before using the PowerQuant® System, ensure that the instrument is calibrated for the PowerQuant® dyes. For more information, refer to the *PowerQuant® System Technical Manual* #TMD047.

1. For the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2, select the *Custom Assays* button on the *Home* screen, or from the *Assays* menu select "Custom Assays".

   For the Applied Biosystems® 7500 Software, Version 2.0.6, and QuantStudio™ Design and Analysis Desktop Software, Version 1.5 and 1.5.1, proceed to Step 2.
2. Open the run template file by selecting "Open" from the *File* menu at the top of the screen. Navigate to the .edt file, and select "Open".
3. Save the .edt template as a .eds file by selecting "Save As…" from the *File* menu.
4. Deselect targets for unused wells.
5. Design a plate map in the PowerQuant® Analysis Software to create an import file with sample names, well position and standard information (See Section 9.H in the *PowerQuant® System Technical Manual* #TMD047 for instructions). Alternatively, to add sample names, select "Plate Setup" from the *Setup* menu, and select the *Define Targets and Samples* tab. Select "Add New Sample", and enter the sample name in the section provided. Repeat for all samples.
6. Navigate to the *Assign Targets and Samples* tab, and highlight the wells that contain replicates of the same sample. Check the *Assign* box adjacent to the corresponding sample name in the *Assign sample(s) to the selected wells* section. Repeat until all samples are assigned.

   Confirm the Run Method.
7. Once samples are assigned properly, save the .eds file.
8. Press the tray door, and place the plate in the open tray door. Push the tray door to close, and immediately press "Start Run".

Evaluating Standard Curves

1. After thermal cycling is complete, select "Analysis" in the left side panel of the Applied Biosystems® 7500 software. Ensure that all wells to be analyzed are highlighted on the *View Plate Layout* tab and that no targets are selected for unused wells.
2. Select "Analyze".
3. For the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2, display the standard curves by selecting "Standard Curve" from the *Analysis* menu. Display the standard curves for all targets by selecting "All" from the *Target* drop-down menu.

   For the Applied Biosystems® 7500 Software, Version 2.0.6, display the standard curves by selecting "Standard Curve" from the *Analysis* menu. Display the standard curve for each target by selecting that target from the *Target* drop-down menu.

   For the QuantStudio™ Design and Analysis Software, select the *Results* tab. Ensure that all wells to be analyzed are highlighted in the *View* tab and that no targets are selected for unused wells. Select "Analyze".

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Exporting Analyzed Data

1. Ensure that all wells with data for export are highlighted in the plate map. Select "Export" from the toolbar.
2. On the *Export Properties* tab of the *Export Data* window, select the following Export Properties:
   - Choose "Results" in the *Select data to export* section.
   - Choose "One File" from the *Select one file or separate files* drop-down menu.
   - Choose ".xls" as the File Type.
   - Specify the appropriate export file name.
   - Use the *Browse* button to select the file location.
   - Select "Start Export".

Data Analysis Using the PowerQuant® Analysis Software

1. Navigate to the downloaded PowerQuant® Analysis Software and click on the icon.
2. Select the *Configuration Settings* icon and confirm that the values for each parameter are appropriate for your analysis.
3. Navigate to the *Import* tab. Confirm that the correct drop-down options for analysis and plate map are selected. Select the *Import* icon.
4. Browse to the .xls file exported from the Applied Biosystems® 7500 Real-Time PCR System or QuantStudio™ Design and Analysis Software, highlight the file and select "OK".
5. Save the file as an Excel® or PDF file to a known location on your computer.
6. Review the PowerQuant® results.

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Additional protocol information is in Technical Manual #TMD047, available online at: **www.promega.com**

