Prime Pro 48 Demonstration Plate

FOR RESEARCH USE ONLY

Techne Part no. PRODEMO48

It is very important to promptly store the kit contents at the temperature specified below to ensure that they perform correctly.

Kit Contents

The Prime Pro 48 demonstration plate is shipped at room temperature. Store it at 2°C to 8°C for up to a month.

For storage times over one month, store the evaluation plate at -15°C to -25°C.

NOTE

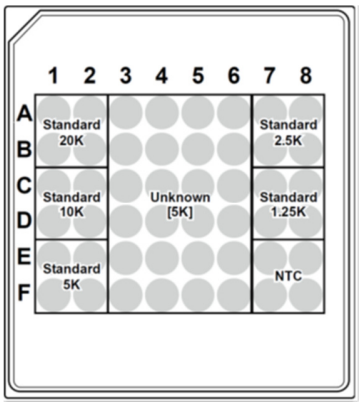
You will need at least 550 µl 2X SYBR Green PCR Master Mix (total 1100ul) purchased from a licensed supplier.

Demonstration Plate

The Prime Pro 48 demonstration plate enables you to test the performance of the Prime Pro 48 Real-Time PCR System. The plate contains PCR primers that are designed to detect and quantify an artificial DNA sequence, with template DNA at defined quantities or no template at all. A standard curve with 20,000, 10,000, 5,000, 2,500, and 1,250 copies is used to quantify an unknown population of 24 replicates.

Setup the Plate

1. Thaw 2X qPCR master mix. Pipette 550µl into a 1.5ml tube.
2. Dilute master mix to 1X by pipetting 550µl DNAse/RNAse free water into the same 1.5ml tube. Vortex briefly to mix.
3. Put the evaluation plate (shown) into the adapter and place it into the centrifuge along with another adapter for balance. Centrifuge at 1000 g for 30 seconds.



4. Move the plate with the adapter onto the Prime Pro 48 dock and turn on the dock light.
5. Remove the aluminum seal from the top of the plate, being careful not to spill any of the plate contents. Tip: Use the squeegee to lift the seal from the corner.
6. Pipette 20µl of the 1X qPCR master mix into each plate well.
7. Remove the white backing from a plate seal.
8. Place the seal on top of the plate, sticky side down, and seal tightly using the squeegee.
9. Place the sealed plate, still inside the adapter, into the centrifuge and add another adapter for balance. Centrifuge at 1000 g for 30 seconds. Make sure no air bubbles are trapped at the bottom of the wells. Recentrifuge if necessary.
10. Place the plate in a dark location at room temperature and leave it for 15 minutes.
11. Vortex the plate for approximately 20 seconds to ensure complete solubilization of the lyophilized reagents.

TIP

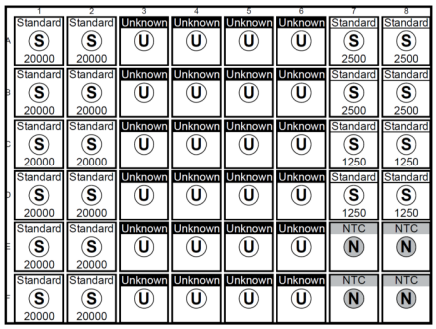
Avoid contaminating the bottom of your plate by holding a clean paper towel between the plate and the vortex head while vortexing.

NOTE

To ensure homogenous re-suspension of primers and template, strictly adhere to the time recommendations in steps 10 and 11.

12. Place the sealed plate, still inside the adapter, into the centrifuge and add another adapter for balance. Centrifuge at 1000 g for 30 seconds.
13. Open the Prime Pro 48 lid by pressing the round silver button on the front to raise its handle, while lifting the handle from the bottom until the Prime Pro 48 pops open.
14. Place the plate into the thermal block inside your Prime Pro 48. Make sure you align the notch on the plate with the indentation in the back left of the block.
15. Close the Prime Pro 48 lid.

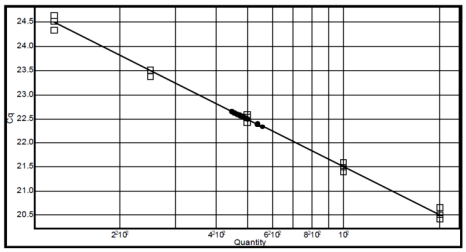
Set Up the Experiment



1. Turn on the netbook and launch the Prime Pro 48 Real-Time PCR software.
2. Click the **Templates** tab on the left.
3. Double click the demonstration plate template file to open an experiment with the appropriate plate layout (shown).
4. Edit the thermal profile as recommended by the master mix supplier.
5. Click **Start** to start the experiment.

Analyze the Results

1. When the experiment finishes open Pro Study software and analyse the data. A plot of the standard curve appears.



2. Check results are within the following guides.

- The **slope** should be between -3.6 and -3.1, corresponding respectively to 90% and 110% efficiency. A slope of -3.32 indicates 100% efficiency, or an exact doubling of template molecules in each PCR cycle.
- The R^2 should be approximately 0.99, indicating a tight correlation between the data points and the standard curve.
- The **standard deviation** of the Cq value for the unknown population should be less than 0.18. Up to four outliers can be removed from the analysis, if necessary.

The demonstration plate is in no way intended to be used for calibration / validation of an instrument or assay, it is purely for demonstration purposes only and should be treated as such.

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