illumina®

Eco™ Evaluation Plate System

FOR RESEARCH ONLY

Illumina catalog # EC-200-1004

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

Kit Contents

The evaluation plate is shipped at room temperature. Store it at 2° to 8°C for up to a month.

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

NOTE

You will need at least 550 µl 2X SYBR Green PCR Master Mix, purchased from a licensed supplier.

Evaluation Plate

The Eco evaluation plate enables you to test the performance of the Eco Real-Time PCR System. The 48-well plate is pre-loaded with primers and template DNA stabilized in each well.

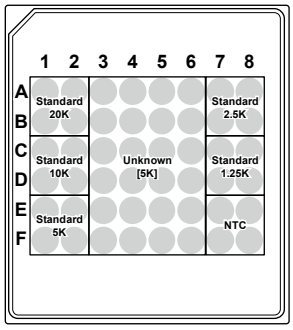
The evaluation plate contains PCR primers that are designed to detect and quantify an artificial DNA sequence, with template DNA at defined quantities or no template at all. A standard curve with 20000, 10000, 5000, 2500, and 1250 copies is used to quantify an unknown population of 24 replicates.

Set Up the Plate

1 Thaw 2X qPCR master mix. Pipette 550 µl into a 1.5 ml tube.

2 Dilute master mix to 1X by pipetting 550 µl DNAse/RNAse-free water into the same 1.5 ml 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 Eco dock and turn on the dock light, if desired.

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 20 µl 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.

10 Place the plate in a dark location at room temperature and leave it for 15 minutes.

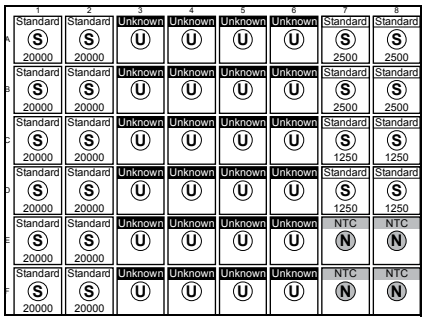
11 Vortex the plate for approximately 20 seconds to ensure complete solubilization of the lyophilized reagents.

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 Place the plate into the thermal silver block on the Eco. Make sure you align the notch on the plate with the indentation in the back left of the block.

14 Close the Eco lid.

Set Up the Experiment



1 Turn on the netbook and launch the Eco Real-Time PCR software.

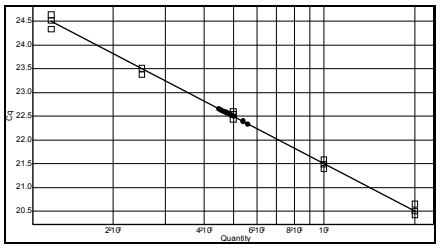
2 Click the **Templates** tab on the left.

3 Double click the EcoEvaluationplate.ecot template file to open an experiment with the appropriate plate layout (shown) and thermal profile.

4 Click **Start** to start the experiment.

Analyze the Results

1 When the experiment finishes, click the **Analyze Data** tab on the left, and then click the **Results** tab at the top.
A plot of the standard curve appears.



2 Check the following values to ensure that the experiment was successful.

- 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 $\mathbf{R^2}$ should be greater than 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 0.167. Up to four outliers can be removed from the analysis, if necessary.

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Complete product documentation is available from our website at http://www.illumina.com/documentation.
An Illumina iCom account login is required. You can request an account at https://icom.illumina.com/Account/Register.