

Protocol Sheet

Applied Biosystems® 7500 (Fast block) real-time PCR run setup instructions for miScript® miRNA PCR Arrays

Important points before starting

- Please read the handbook supplied with the miScript miRNA PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.
- Please make sure the real-time PCR instrument is working properly. Refer to the manufacturer's Installation and Maintenance manual if needed.

Procedure

Creation of PCR protocol template

1. Open the ABI 7500 v2.0.4 software on the desktop of the computer that is connected to the ABI 7500 instrument.
2. Select "File" > "New Experiment" > "Advanced Setup."
3. Under "Setup" > "Experiment Properties" menu (see Figure 1)
 - Select "7500 Fast (96 Wells)."
 - Select "Quantitation – Standard Curve."
 - Select "SYBR® Green Reagents."
 - Select "Standard (~ 2 hours to complete a run)."



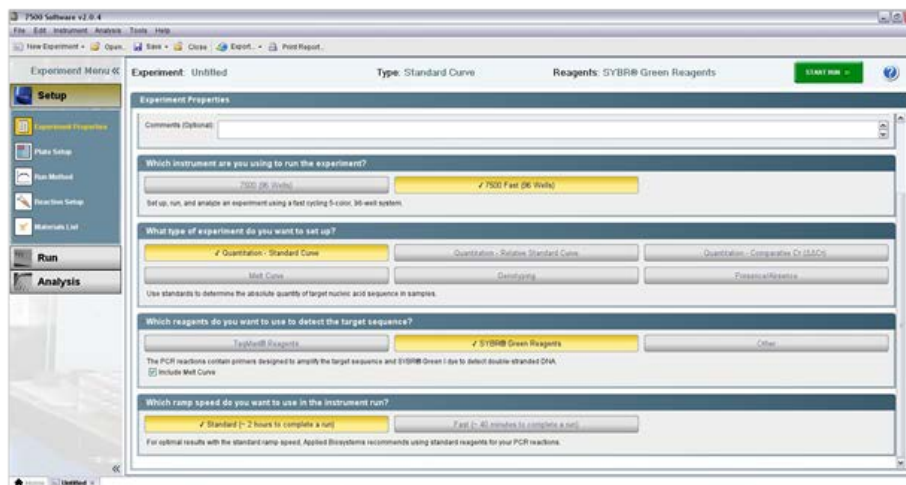


Figure 1. Experiment properties.

4. Under “Setup” > “Plate Setup” menu

- In “Define Targets and Samples” tab, ensure that “SYBR” is listed beside “Target 1.”
- In “Assign Targets and Samples” tab:
 - Highlight the entire plate in the “View Plate Layout” window.
 - Check the “Assign” box next to “Target 1” under “Assign targets to the selected wells” and verify that all wells in “Plate Layout” view have the “U” symbol (“U” = unknown).

5. Under “Setup” > “Run Method” menu (See Figure 2).

- Enter 25 μ L for “Reaction Volume Per Well”
- Adjust parameters to reflect the following
 - Holding Stage
 - Temperature: 95°C
 - Time: 15:00
 - Cycling Stage (3 Steps)
 - Number of Cycles: 40
 - Step 1: 94°C, 00:15
 - Step 2: 55°C, 00:30
 - Step 3: 70°C, 00:34, Data Collection On
 - Melt Curve Stage
 - Ensure “Continuous” is checked
 - Data Collection On during ramp from 60°C to 95°C

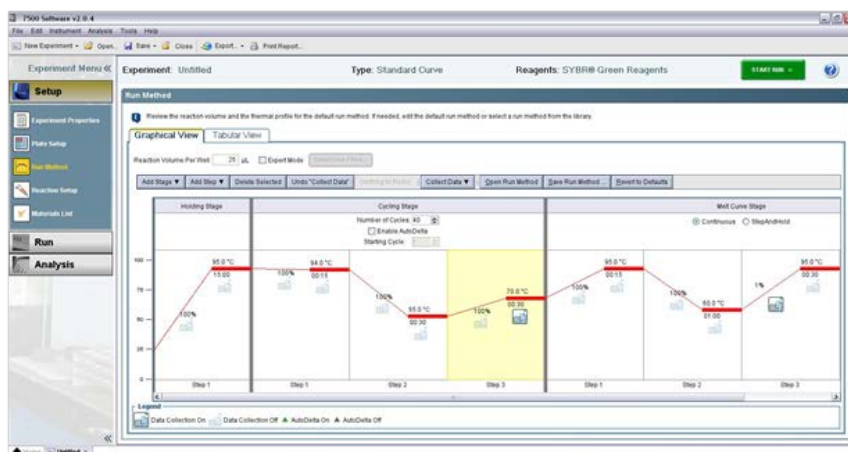


Figure 2. Run Method.

6. Select "File" > "Save As Template." Save the file as Experimental Template files (*.edt) with the filename "miScript_miRNA_PCR_Array_Template" (click "Save").

Note: Alternatively, download the PCR protocol template file (miScript_miRNA_Template_ABI7500_M) and plate setup template file (miScript_miRNA_iCycler_Template_Plate.pts) from the QIAGEN website, www.qiagen.com.

Performing real-time PCR detection

7. If the thermocycler is off, press the power button to switch on the instrument. Wait for the instrument to boot and display the "Power" status light. Switch on the computer connected to the thermocycler.
8. Ensure that the plate has been centrifuged for 1 min at 1000 g to remove any bubbles.
9. Open the tray and place the plate in the precision plate holder with the last row (row H) facing front. Make sure the plate is properly aligned in the holder, well A1 should be positioned at the top-left corner of the tray. To close the tray door, press the tray to move it into the instrument while applying pressure to the right side of the tray at an angle.
10. Open the ABI 7500 v2.0.4 software.
11. Select "File" > "New Experiment" > "From Template." Select the "miScript_miRNA_PCR_Array_Template" file and click "Open."
12. Verify that run method is correct.
13. Select "Run Menu."
14. Click "Start Run" in "Run Status" window.

After the PCR run

15. To determine C_T values, set **Baseline** and **Threshold** as described in the *miScript miRNA PCR Array Handbook*.
16. Export C_T values.

The miScript miRNA PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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