Protocol Sheet

ABI StepOnePlus® (for Software Version 2.0) instrument setup instructions for RT² Profiler PCR Arrays

Important points before starting

- Please read the handbook supplied with the RT² Profiler PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Procedure

1. **Open the ABI StepOnePlus software on the desktop of the computer that is connected to the ABI StepOnePlus system.**
2. **Select New Experiment on the Upper Toolbar.**
3. **Define: Experiment Properties**
    - **Label your experiment**
        - **Type in Experiment Name**
        - **Type in Barcode, Username, Comments (optional)**
    - **Select Instrument**
        - **StepOnePlus Instrument (96 wells)**
    - **Select Experiment Type**
        - **Quantitation**
    - **Click Next on bottom of screen**
4. **Define: Methods and Materials**
    - **Quantitation method**
        - **Standard Curve**
    - **Reagents to detect target sequence**
        - **SYBR® Green reagents**
        - **Keep "Melt Curve" checked**
    - **Ramp speed**
        - **Standard (about 2 hours to complete a run)**
    - **Template type**
        - **cDNA (complementary DNA)**
    - **Click Next on bottom of screen**
5. **Set up: Targets**

- **How many targets do you want to quantify?**
  - **1**
- **Uncheck: SetUp Standards**
  - **Target Name:**
    - **Target 1**
  - **Reporter**
    - **SYBR**
  - **Quencher**
    - **None**
- **Click Next on bottom of screen**
- **Ignore Warning, click OK**

6. **Set up: Standards**
   - **How many points?**
     - **2**
   - **How many replicates?**
     - **1**
   - **Click Next on bottom of the screen**
7. **Set up: Samples**
   - **How many samples?**
     - **96**

     Note: If your instrument does not recognize all 96 wells, see additional instructions on the final page
   - **How many replicates?**
     - **1**
   - **How many negative controls?**
     - **0**
   - **Which sample/target reactions do you want to set up?**
     - **Select: All Sample/Target Reactions**
   - **Verify al wells in Plate Layout view have the "U" symbol (U=unknown)**
   - **Click Next on bottom of screen**
8. **Set up: Run Method**
   - **This setting should default to run protocol with melting curve**
     - **Verify Capture Data icon is present at**
       - **Cycling stage: 60°C (1 minute step)**
       - **Melting Curve stage: during ramp from 60°C to 95°C**

- **Set Reaction Volume to 25 µl**
- **Verify Number of Cycles is set to 40**

9. **Click "Finish Designing Experiment"**
10. **Ignore Warning**
11. **Click OK when prompted "You did not set up standards on the plate"**
12. **Load your plate into the instrument**
13. **Start Run for this experiment**
14. **Save your experiment before starting the run**

Note: For customers whose instruments do not recognize all 96 wells of the array, please use the following instructions.

ABI StepOne Plus — Modified Setup

15. **Open the ABI StepOnePlus software on the desktop of the computer that is connected to the ABI StepOnePlus system.**
16. **Select Advanced Setup**
17. **Define: Experiment Properties**
    - **Label your experiment**
      - **Type in Experiment Name**
      - **Type in Barcode, User Name, Comments (Optional)**
    - **Select instrument**
      - **StepOnePlus Instrument (96 wells)**
    - **Select experiment type**
      - **Quantitation — Standard Curve**
    - **Select reagents**
      - **SYBR Green**
    - **Select ramp speed**
      - **Standard (about 2 hours to complete)**

18. **Click Plate Setup (on left)**
    - **Click Assign Targets and Samples**
        - **Highlight entire plate**
        - **Check the box next to Target 1 under Assign Targets to the Selected Wells**
        - **Verify that all wells in Plate Layout view have the U symbol (U=unknown)**
19. **Click Run Method (This setting should default to run protocol with melting curve)**
    - **Verify Data Capture icon is present at:**
        - **Cycling Stage: 60°C (1 minute step)**
        - **Melting Curve Stage: During ramp from 60°C to 95°C**
    - **Set Reaction Volume to 25 µl**
    - **Verify Number of Cycles is set to 40**
20. **Click Start Run**

The RT^2 Profiler PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature . Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx .

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