

ABI StepOnePlus (for Software Version 2.0)

Instrument Setup Instructions for RT²Profiler™ PCR Arrays

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
 - a. Label Your Experiment
 - i. Type In Experiment Name
 - ii. Type In Barcode, User Name, Comments (Optional)
 - b. Select Instrument
 - i. StepOnePlus Instrument (96 wells)
 - c. Select Experiment Type
 - i. Quantitation
 - d. Click Next on Bottom of the Screen
4. Define: Methods & Materials
 - a. Quantitation Method
 - i. Standard Curve
 - b. Reagents to Detect Target Sequence
 - i. SYBR Green Reagents
 - ii. Keep 'Melt Curve' Checked
 - c. Ramp Speed
 - i. Standard (~ 2 hours to complete a run)
 - d. Template Type
 - i. cDNA (complementary DNA)
 - e. Click Next on Bottom of the Screen
5. Set Up: Targets
 - a. How Many Targets Do You Want to Quantify?
 - i. 1

- b. Uncheck: SetUp Standards
 - i. Target Name:
 - 1. Target 1
 - ii. Reporter
 - 1. SYBR
 - iii. Quencher
 - 1. None
 - c. Click Next on Bottom of the Screen
 - d. Ignore the Warning – Click OK
6. Set Up: Standards
- a. How Many Points?
 - i. 2
 - b. How Many Replicates?
 - i. 1
 - c. Click Next on Bottom of the Screen
7. Set Up: Samples
- a. How Many Samples?
 - i. 96
 - 1. **NOTE: If you instrument is not recognizing all 96 wells, please see additional instructions on the last page.**
 - b. How Many Replicates?
 - i. 1
 - c. How Many Negative Controls?
 - i. 0
 - d. Which Sample/Target Reactions Do You Want To Set Up?
 - i. Select: ALL Sample/Target Reactions
 - e. Verify all wells in Plate Layout view have the “U” symbol (“U” = unknown)
 - f. Click Next on Bottom of the Screen
8. Set Up: Run Method

Technical Note

- a. This setting should default to run protocol with melting curve.
 - i. Verify Data Capture icon is present at
 1. Cycling Stage: 60°C (1 minute step)
 2. Melting Curve Stage: During ramp from 60°C to 95°C
 - b. Set Reaction Volume to 25 ul
 - c. 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 those customers whose instruments do not recognize all 96-well of the PCR Arrays, please use the instructions on the next page.

ABI StepOnePlus – Modified Setup

1. Open the ABI StepOnePlus software on the desktop of the computer that is connected to the ABI StepOnePlus system.
2. Select Advanced Setup
3. Define: Experiment Properties
 - a. Label Your Experiment
 - i. Type In Experiment Name
 - ii. Type In Barcode, User Name, Comments (Optional)
 - b. Select Instrument
 - i. StepOnePlus Instrument (96 wells)
 - c. Select Experiment Type
 - i. Quantitation-Standard Curve
 - d. Select Reagents
 - i. SYBR Green
 - e. Select Ramp Speed
 - i. Standard (~2 hours to complete)
4. Click Plate Setup (on left)
 - a. Click Assign Targets and Samples Tab
 - i. Highlight the entire plate
 - ii. Check the box next to Target 1 under Assign targets to the selected wells
 - iii. Verify all wells in Plate Layout view have the “U” symbol (“U” = unknown)
5. Click Run Method (This setting should default to run protocol with melting curve.)
 - a. Verify Data Capture icon is present at:
 - i. Cycling Stage: 60°C (1 minute step)
 - ii. Melting Curve Stage: During ramp from 60°C to 95°C
 - b. Set Reaction Volume to 25 uL
 - c. Verify Number of Cycles is set to 40
6. Click Start Run