invitrogen

# *ProQuantum™ Technical Guide*

## USER GUIDE

**Publication Number** MAN0017048

**Revision** B.0

**For Research Use Only. Not for use in diagnostic procedures.**

Thermo Fisher
SCIENTIFIC

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:** Revision history of Pub. no. MAN0017048

| Revision | Date | Description |
|---|---|---|
| B.0 | 3 November 2021 | The manufacturer address was updated. |
| A.0 | 6 November 2017 | New manual for ProQuantum™ Immunoassay Kits. |

**Important Licensing Information**: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**Trademarks**: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

# Contents


- Product information ........ 5
  - Product description ........ 5
  - Contents and storage ........ 5
  - Workflow ........ 6
- Methods ........ 7
  - General guidelines ........ 7
    - Guidelines for handling reagents ........ 7
    - Guidelines for working with small volumes ........ 8
    - Guidelines for preparing and calculating standard curves ........ 8
  - Required materials not provided ........ 9
  - Prepare reagents ........ 9
    - Prepare serum samples ........ 9
    - Thaw reagents ........ 10
    - Reconstitute standard ........ 10
    - Prepare working plate (on cold block 4°C) ........ 10
  - Run assay ........ 13
    - Bind analyte (1 hour) ........ 13
    - Perform qPCR ........ 13
  - Limited product warranty ........ 14
- **APPENDIX A** Troubleshooting ........ 15
- **APPENDIX B** Instrument settings ........ 17
  - Determine ramp rate (QuantStudio™ instruments only) ........ 17
  - Settings for QS 3 and 5 Systems ........ 19
    - Set up experiment properties ........ 19
    - Assign targets and samples ........ 20
    - Define Method ........ 21
  - Settings for the QS 12K System ........ 22
    - Set up experiment properties ........ 22
    - Define target ........ 23
    - Assign wells ........ 24
    - Define Method ........ 25

Settings for QS 6 and 7 Systems . . . 26
- Set up experiment properties . . . 26
- Define target . . . 27
- Assign wells . . . 28
- Define Method . . . 29

Settings for the StepOnePlus System . . . 30
- Set up experiment properties . . . 31
- Define targets and samples . . . 31
- Assign targets and samples . . . 32
- Define Method . . . 33
- Importing .csv files . . . 34

Settings for the 7500 System . . . 35
- Set up experiment properties . . . 36
- Define targets and samples . . . 36
- Assign targets and samples . . . 37
- Define Method . . . 38

Settings for non-AB instruments . . . 39
- Importing .csv files . . . 40

# Product information

## Product description

ProQuantum™ high-sensitivity immunoassays are designed to detect and quantify target-specific proteins using qPCR as a readout. The assay has a large dynamic range with high sensitivity and uses small sample volumes. The workflow is steamlined with no wash steps. Due to the pushing the boundaries for high sensitivity, no wash, no shaking assay with minimal volumes, it is important to pay close attention to pipetting accuracy and mixing steps to achieve good results.

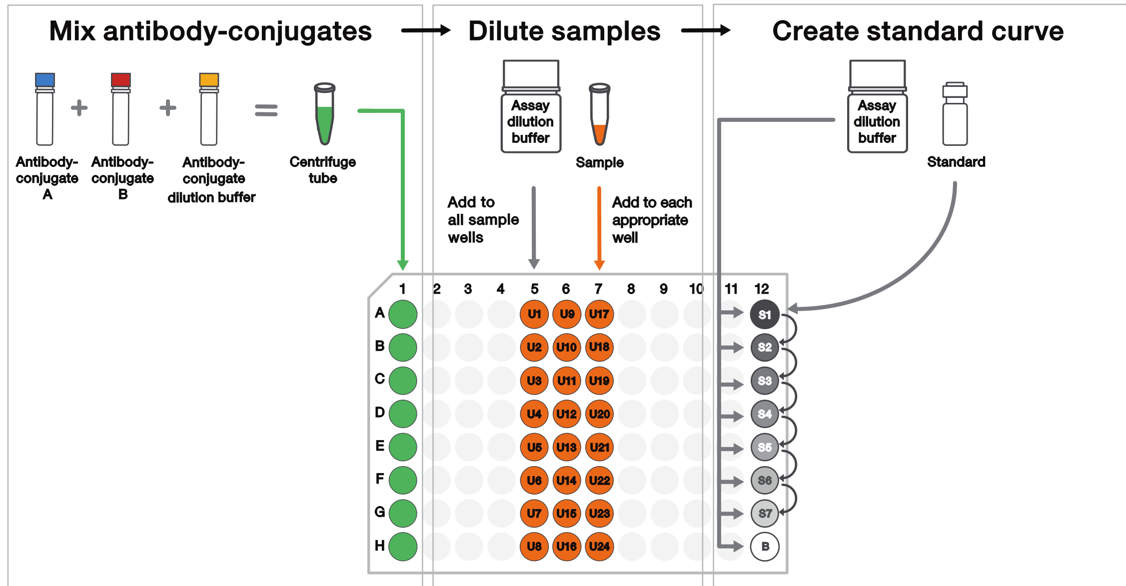
## Contents and storage

ProQuantum™ immunoassay kits are shipped frozen on dry ice. Upon receipt, store the kits at –20°C. Do not store at –80°C.

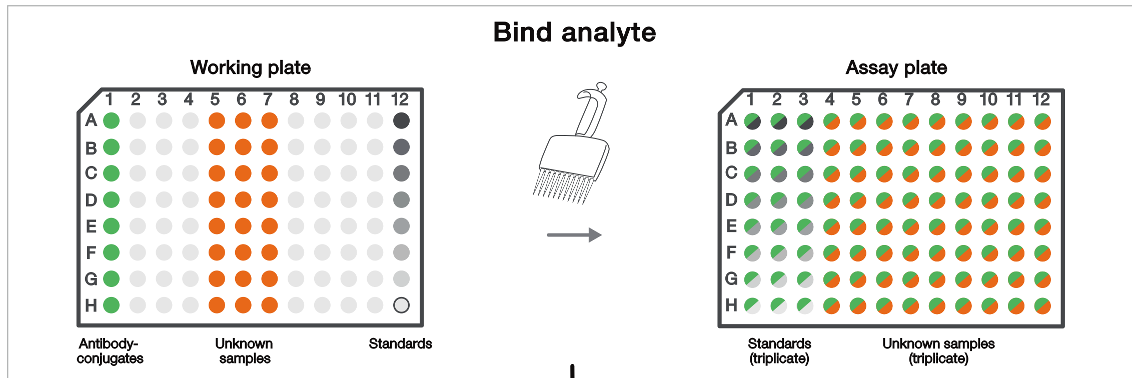
Each kit comes with all the reagents necessary to perform the assay.

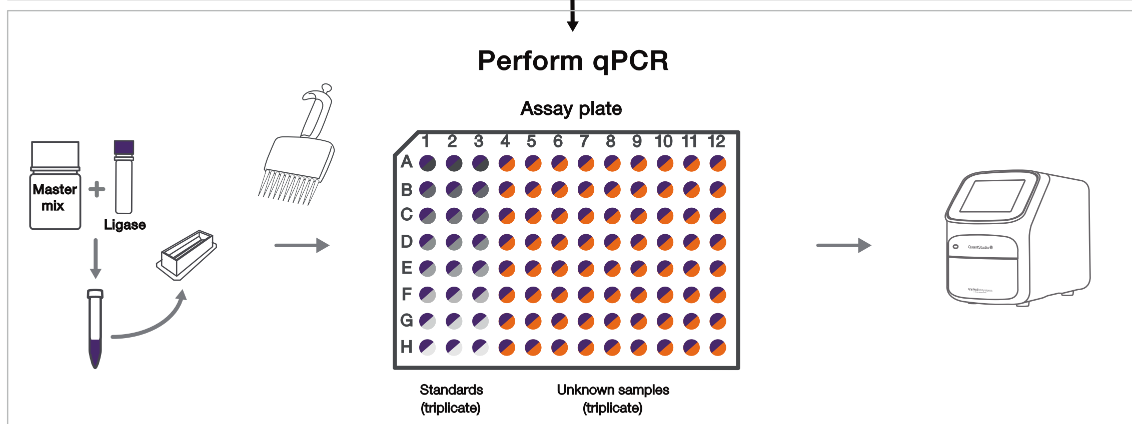
# Workflow

## Prepare working plate




## Run assay



# Methods

## General guidelines

- The ProQuantum™ immunoassay is a DNA assay, and will not perform properly if samples contain inhibitors that affect such assays (e.g., DNA ligase inhibitors).
- Wear gloves, use DNase/RNase/pyrogen free plastic ware, and practice proper DNA handling techniques.
- Use a plate seal hand tool to ensure complete adherence to avoid any evaporation or contamination.
- Use best practices with pipetting to minimize CV.
- Use a working plate to prepare reagents and a multi-channel pipette when transferring reagents to the assay plate to minimize handling time and thus, evaporation.
- Use a plate rack to hold the assay plate to avoid splashing when unsealing the adhesive seals.
- Outliers are more readily identified if the assay is run in triplicate.
- Be sure to use appropriate PCR plates that are compatible with the qPCR instrument and block type (See **thermofisher.com/plastics** for compatibility details).
- Do not vortex plates.
- Mixing of the reagents is critical to the assay. Mixing can be performed by careful pipetting up and down 10 times, or by striking the side of the plate against the palm of your hand or a stationary object like the lab bench. The strike should be hard enough to cause liquid to splash to the top of the adhesive seals and to the other side within the well.

### Guidelines for handling reagents

- Centrifuge vials before pipetting to ensure the contents are at the bottom of the tube.
- **Do not thaw Ligase**. Keep Ligase at –20C or on ice at all times. Ligase is viscous. Careful with pipetting accurate amounts and not cause bubbles.
- Thaw all reagents **except Ligase** at room temperature.
- Larger vials like Assay Dilution Buffer may take awhile to thaw, and can be thawed in a warm water bath.
- Keep Ligase and thawed reagents on ice.
- Use a refrigerated cold block for the working plate to keep all reagents cool during preparation steps. If a cold block is not available, keep the working plate on ice.
- If any particulate matter is present in the sample, centrifuge or filter sample before performing the assay.

## Guidelines for working with small volumes

- When working with small volumes, good practices are critical for the best CVs.
- Make sure pipettes are calibrated. The appropriate size pipettes are used such as 2 µL or 20 µL. When possible, the use of a multi-channel pipette can make the workflow steps easier and minimize CVs.
- Use low retention filter tips.
- The plate seals must adhere tightly, especially around the edge of the plate using the plate seal hand tool. This will prevent any evaporation as well as enabling mixing of the plate without contamination between wells.
- Use low dead volume troughs designed for smaller volumes to aid use of multi-channel pipettes.

## Guidelines for preparing and calculating standard curves

- Ensure that the reconstitution and serial dilution calculation setup and steps was performed properly.
- Make sure that the reconstitution of the standard protein was correct.
- Make sure that there is adequate mixing during the serial dilution steps and that the pipette tips were disposed of at each step.
- Ensure that the standard curve range was set within the range of the extended standard curve example in the protocol.
- If desired, extend the range of the standard curve by modifying the **Rate of dilution**, or **S1 concentration** when setting up the Method in the ProQuantum™ software.

- Review the standard curve data for any outliers using the ProQuantum™ software. The default setting for outlier detection flags any value that is outside of 70–130% standard recovery or 15% CV of the replicate data.

# Required materials not provided

| Product | Cat. No. [1] |
| --- | --- |
| Calibrated pipettes and low retention filter tips | MLS |
| 96-well plate cold block | Fisher 50-589-601 |
| 96-well assay plate and 96-well working plate | MLS |
| 25-mL reagent reservoir × 2 | Fisher 14387071 |
| Microtiter plate adhesive seals × 2 | Fisher 4306311 |
| Microtiter plate adhesive seal, optical grade | Fisher AB1170 |
| Plate seal hand tool | 4333183 |
| Centrifuge with swinging bucket rotor for 96-well plates, and microcentrifuge | MLS |
| 96-well plate rack | Fisher 05-541-80 |
| RNase-free 1.5-mL microcentrifuge tube | AM12400 |
| Sterile 15-mL conical tube | 339651 |

[1] MLS: major laboratory supplier.

# Prepare reagents

## Prepare serum samples

Maintain the samples at 2–8°C during handling.

1. Collect whole blood in a covered test tube.
2. Allow the collected blood to clot by leaving it undisturbed at room temperature (usually 15–30 minutes).
3. Centrifuge at 1000–2000 × *g* for 10 minutes in a refrigerated centrifuge to separate the clotted material.
4. Immediately transfer the resulting supernatant (serum) into a clean polypropylene tube using a Pasteur pipette.
5. If the serum is not analyzed immediately, apportion the serum into 0.5 mL aliquots. Store and transport the aliquots at –20°C or lower.

## Thaw reagents

1. Thaw all reagents **except Ligase** at room temperature.
2. Keep Ligase and thawed reagents on ice.

## Reconstitute standard

1. Reconstitute one standard vial with Assay Dilution Buffer. See label on the vial for the reconstitution volume. Mix by gently inverting the vial five times. **Do not vortex**.

   **Note:** Do mix by pipetting because the crystallized powder can be trapped in the pipette tip. If the powder does not go into solution after a few minutes, repeat the inversion.

2. Incubate for 15 minutes at room temperature.

## Prepare working plate (on cold block 4°C)

### Mix antibody-conjugates

The volumes provided in the following table are sufficient to run an entire 96-well plate using a 50 µL reaction volume. The volumes can be scaled proportionally depending upon the number of assay wells being used and the reaction volume. If using 20 µL reaction volumes, 2 µL of antibody-conjugate mixture is required for each assay well. If using 50 µL reaction volumes, 5 µL of antibody-conjugate mixture is required for each assay well.

1. Add the following components to a 1.5-mL microcentrifuge tube, then mix by pipetting up and down.

| Component | Volume |
|---|---|
| Antibody-conjugate A | 12 µL |
| Antibody-conjugate B | 12 µL |
| Antibody-conjugate Dilution Buffer | 696 µL |

2. Dispense ≥80 µL of antibody-conjugate mixture to each well in one column of the working plate.

   **Note:** This step may not be needed if only running partial plates or not using a multi-channel pipette.

## Dilute samples 10-fold

Sample dilution is performed directly in the working plate.

1. Add the volumes of sample and Assay Dilution Buffer in each assay well of the working plate.

| Component | 20 µL reaction | 50 µL reaction |
|---|---|---|
| Assay Dilution Buffer | 18 µL | 45 µL |
| Unknown sample | 2 µL | 5 µL |

2. Mix the contents of the assay wells by pipetting up and down.

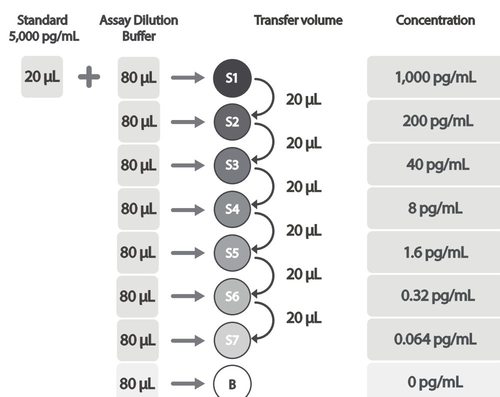
## Create standard curve

1. Add 80 µL of Assay Dilution Buffer to each well in one column of the working plate that is designated for standards.

2. Mix reconstituted standard by gently inverting the vial five times. Transfer 20 µL of the protein standard to the first well (S1), then mix.

**Note:** The total volume in protein standard wells is 100 µL in order to minimize the CV for standard curve calculation.

3. Make serial dilutions of the standard by transferring 20 µL from well S1 to well S2. Continue serial dilution to well S7. The Background well (B) should only contain Assay Dilution Buffer. Mix wells thoroughly. Change pipette tips between dilution steps.




**Note:** The standard curve can be modified to extend the range. See “Guidelines for preparing and calculating standard curves” on page 8.

4. Seal the working plate with an adhesive seal using the plate seal hand tool.

5. Mix by striking the side of the plate against the palm of your hand three times. Rotate the plate and strike the other side of the plate against your palm three times.

6. Centrifuge at 3,000 × *g* for 1 minute to collect the liquid at the bottom of all wells.

7. Discard remaining unused reconstituted standard.

# Run assay

## Bind analyte (1 hour)

Keep the assay plate on a plate rack except during mixing or centrifugation steps.

1. Use a multichannel pipette to transfer the volume of antibody-conjugate mixture appropriate for your reaction volume from the working plate to all assay wells in the assay plate.

| 20 µL reaction | 50 µL reaction |
|---|---|
| 2 µL | 5 µL |

2. Transfer the volume of standards or diluted samples appropriate for your reaction volume from the working plate to the appropriate wells in the assay plate. Mix thoroughly by pipetting up and down several times.

| 20 µL reaction | 50 µL reaction |
|---|---|
| 2 µL | 5 µL |

3. Seal the assay plate with an adhesive seal. Mix by striking the plate as previously described. Centrifuge at 3000 × *g* for 1 minute.
4. Incubate the assay plate for 1 hour at room temperature, or if desired, overnight at 4°C.

## Perform qPCR

1. Add 5 mL of Master Mix and 30 µL of Ligase to a 15-mL conical tube. Mix thoroughly by pipetting up and down, then pour the qPCR reaction mixture into a reagent reservoir.

**Note:** These volumes are sufficient to run an entire 96-well plate using a 50 µL reaction volume. The volumes can be scaled proportionally depending upon the number of assay wells being used and the reaction volume.

2. Add the volume of qPCR reaction mixture appropriate for your reaction volume to all assay wells. Mix by pipetting up and down. Avoid introducing bubbles in the wells.

| 20 µL reaction | 50 µL reaction |
|---|---|
| 16 µL | 40 µL |

3. Seal the assay plate with an optical plate seal. Ensure there is complete adhesion using the plate seal hand tool.
4. Mix by striking the plate as previously described, then centrifuge at 3,000 × *g* for 1 minute.

5. Input qPCR instrument settings.

| Parameter | Settings for Applied Biosystems™ instruments [1] |
|---|---|
| Experiment type | Standard Curve or Quantitation - Standard Curve |
| Reagents | TaqMan™ reagents |
| Reporter dye | FAM |
| Quencher | NFQ-MGB [2] |
| Passive reference | ROX |
| Assign wells | Define all wells of the 96-well plate as Unknown |
| Threshold | 0.2 |
| Baseline | 3–15 |

[1] For non-Applied Biosystems instruments, collect Ct values for each data point using the equivalent settings in the table.
[2] For instruments without this option, enter "None" or "Non-fluorescent".

6. Run the PCR plate using the protocol conditions appropriate for the block type.

| Step | Temp (°C) | Time | | Stage |
|---|---|---|---|---|
| | | Standard Protocol | Fast Protocol[1] | |
| Ligation | 25 | 20 min | 20 min | Hold |
| Ligase inactivation | 95 | 2 min | 2 min | Hold |
| Denaturation | 95 | 15 s | 1 s | 40 cycles |
| Annealing/ extension | 60 | 1 min | 20 s | |

[1] Use default settings for 7500, 7500 Fast, 7900HT, or non-Applied Biosystems instruments (e.g., 3 second denaturation step and 30 second annealing/extension step).

7. Import .eds, .sds., or .cvs files to the ProQuantum™ software (available at **apps.thermofisher.com/apps/proquantum**) to generate standard curves and determine sample concentrations.

**Note:** For non-Applied Biosystems instruments, see "Settings for non-AB instruments" on page 39 for details on handling .cvs files.

# Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

# Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| No Ct values in data file | qPCR software was not set up properly. | Make sure that all 96 wells are designated as unknown. |
| | | Make sure that the parameters including FAM, ROX passive was set. |
| | | Make sure the camera collected is at the last cycle point. |
| No data collected error ProQuantum Software - error message that no data was collected | File was saved to a USB memory device directly from the instrument and not from the connected PC. | Go back to the computer and locate the file and save it from the computer and not directly from the instrument. |
| Poor standard curve (poor recovery) | Improper serial dilution. | Verify that amounts of Assay Dilution Buffer and recombinant protein is correct for each well. |
| | | Verify that the range of dilutions are within the recommended range in the kit protocol. |
| | Contamination from well to well. | Make sure that the plates are sealed tightly so that no spillage happens during mixing and plate centrifugation. |
| | | Make sure to change pipette tips in between wells or samples. |
| Poor standard curve (high CV) | Pipetting issue. | Verify that the pipettes are calibrated. |
| | | Make sure to use low retention filter tips, which are important for small volume pipetting. |
| | | Make sure that bubbles are minimized when pipetting up and down. |
| | | Use best practices with pipetting, i.e., pipette liquid onto the side of the plate, visual examination. |
| | | Use multi-channel pipettes where possible. |
| | | Ensure that each pipette tip is tightly secured with the visually correct amount of liquid. |
| | | Do not do reverse pipetting with small volumes. |
| | | If small volume such as 2 µL is a problem with consistency, try increasing the volume to 5 µL. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Poor standard curve (high CV) *(continued)* | Improper mixing. | Verify that the plate is sealed properly using a hand tool. |
| | | When mixing, make sure it is thoroughly done (i.e., pipette up and down 10 times or striking the plate hard enough so that liquid goes from one side of the well to the other) at all steps when introducing new components. |
| | | Make sure that centrifugation of the plate occurs after the mixing step to ensure all reagents come to the bottom of the plate. |
| | Evaporation when using small volumes. | Minimize any setup time by using the working plate or another method so that small volumes are not exposed for long periods of time. |
| | Insufficient replicates. | Run samples in triplicate so that outliers are more easily identified. |
| Poor standard curve (high low end CV)<br>High CV only at the low end of the curve but not the linear portion of the curve | The assay is at the limit of sensitivity. | Acceptable data is when the CV is less than 20%, so data cannot be reliable in this range. |

# Instrument settings

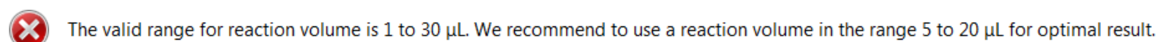
Settings for running ProQuantum™ immunoassays on Applied Biosystems™ qPCR instruments are provided in the following section.

On Applied Biosystems™ instruments, create a Method with the parameters (instrument settings, desired reaction volume, and PCR conditions) appropriate for your specific instrument. This Method can be saved as a template, and reused on any future runs that share identical parameters.

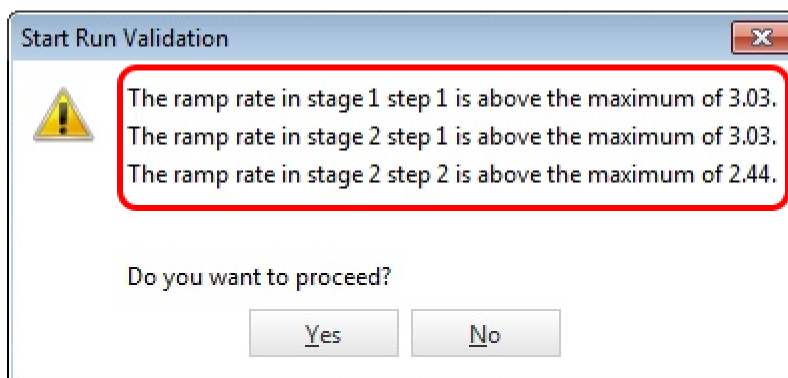
## Determine ramp rate (QuantStudio™ instruments only)

When using a 50 µL reaction volume with a QuantStudio™ instrument, determine the ramp rate to be used for defining the Method.

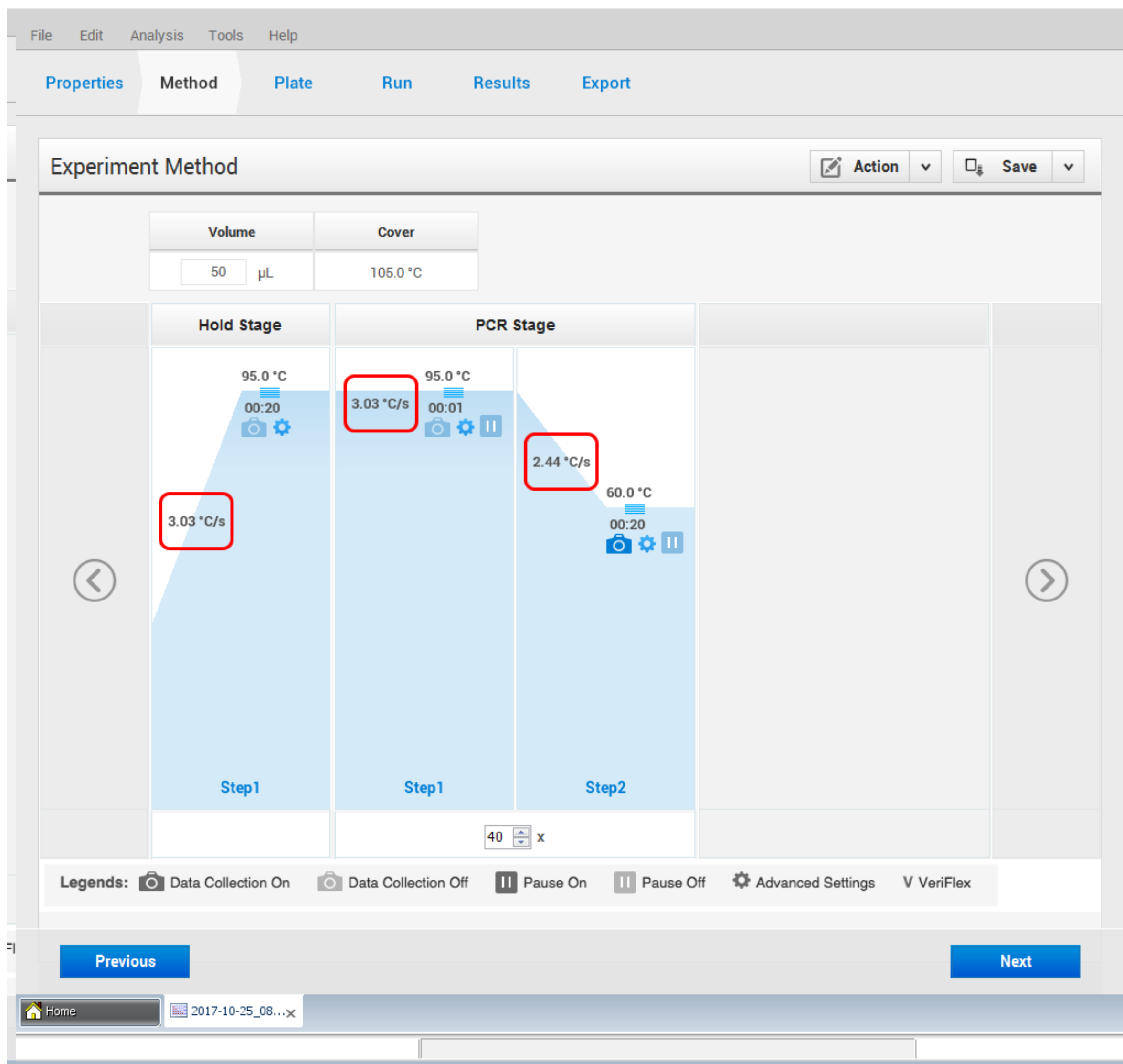
1. Go the **Run** screen and change the reaction volume to 50 µL.
2. An error message will display. Click **OK**.




3. Click **Start run**.
4. A dialog box will appear and display the maximum ramp rate for each step for the volume. Copy the ramp rate values to use in defining the method.
5. Click **No**.

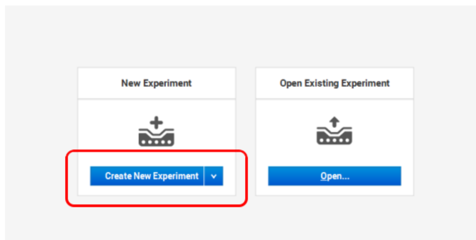


6. Edit the ramp rate when creating your Method with the appropriate values taken from the instrument.



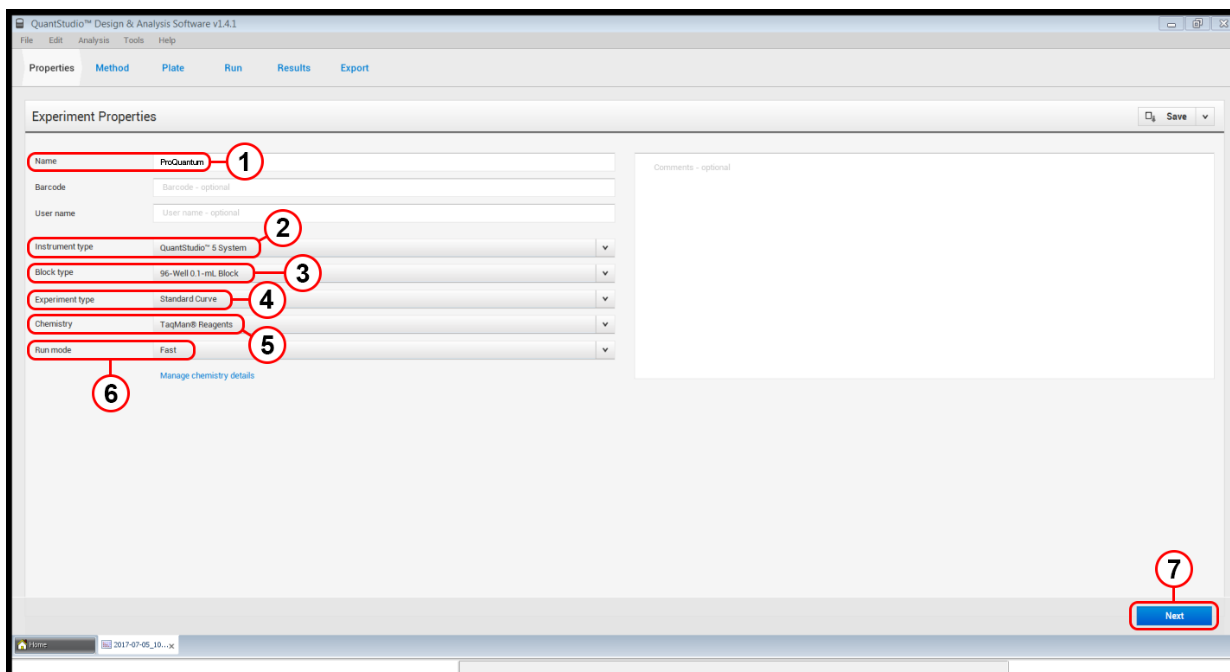
# Settings for QS 3 and 5 Systems

Settings for the QuantStudio™ 3 System and QuantStudio™ 5 System can be modified from the **New experiment** screen.



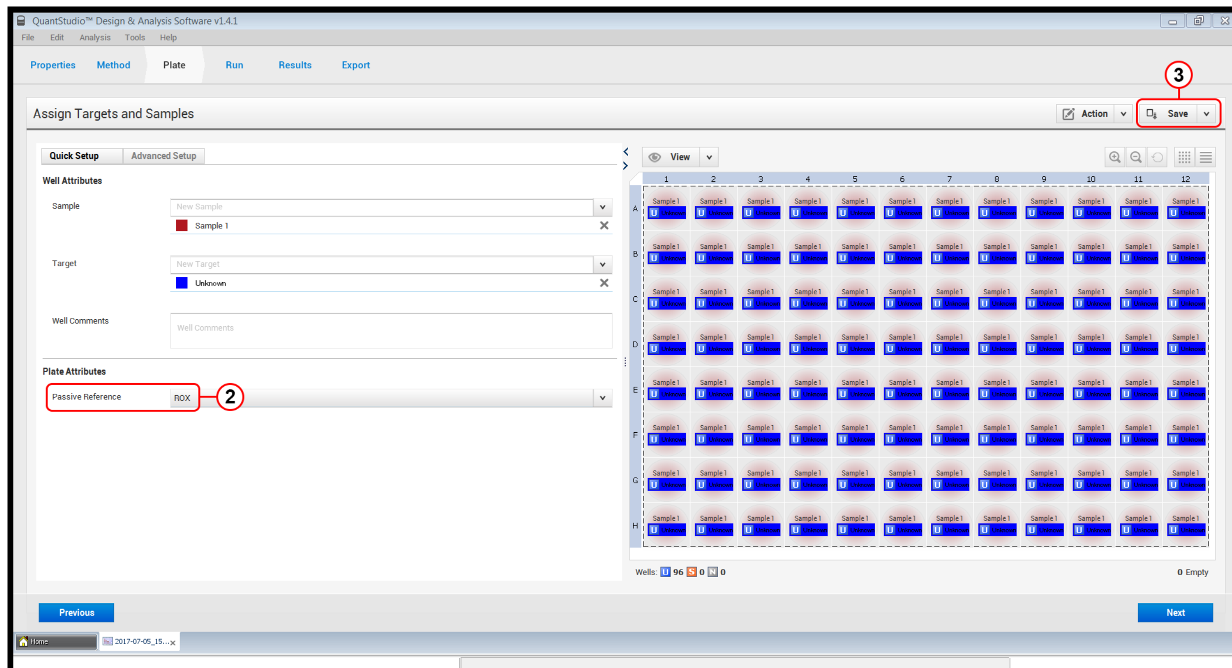

## Set up experiment properties

1. Enter "ProQuantum" as the **Experiment name**.
2. Select your instrument type (QuantStudio™ 3 System or QuantStudio™ 5 System).
3. Select your block type (0.1 mL or 0.2 mL).
4. Ensure experiment type is set to **Standard curve**.
5. Ensure chemistry is set to **TaqMan™ Reagents**
6. Select the appropriate run mode based on your block type (**Fast** for 0.1 mL, or **Standard** for 0.2 mL).
7. Click **Next**, and proceed to next step.



## Assign targets and samples

1. Assign all wells as unknown by setting wells to **U**.
2. Ensure passive reference is set to **ROX**.
3. Save Method.



## Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

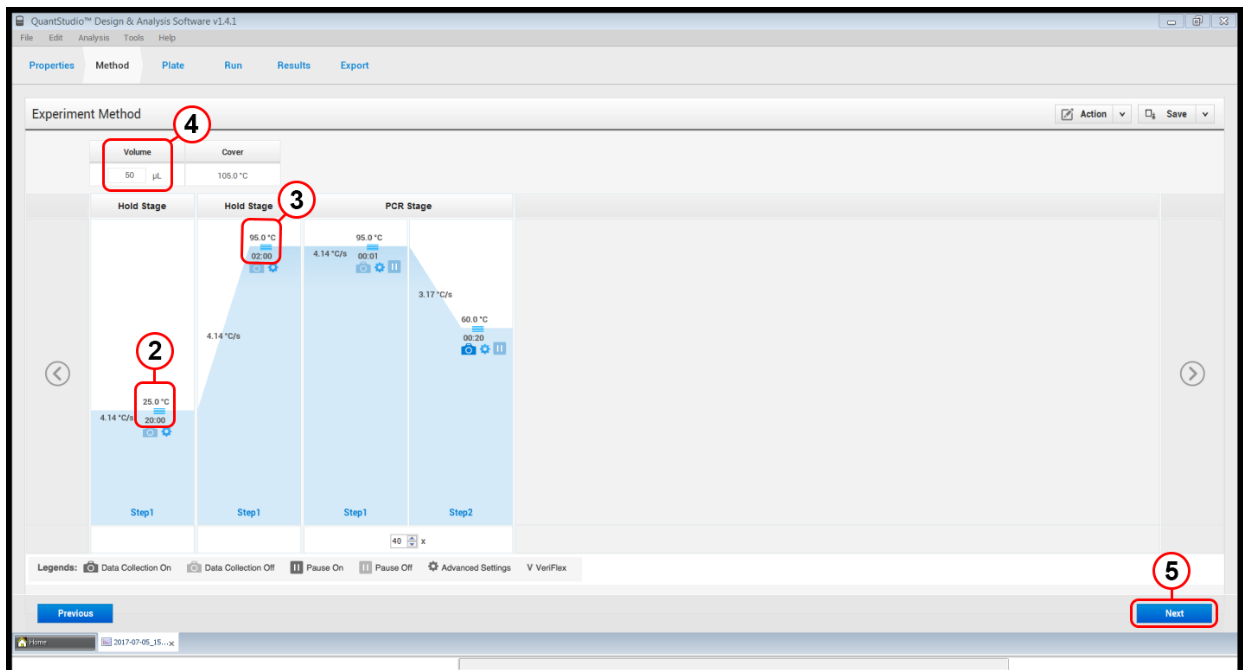
1. Add a new **Hold stage**.
2. Set first **Hold stage** to 25°C for 20:00.
3. Modify second **Hold stage** to 95°C for 2:00.
4. Set **Reaction volume per well** to 20 µL or 50 µL depending upon the protocol to be performed.

---

**Note:** The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio™ instruments only)" on page 17 for details.)

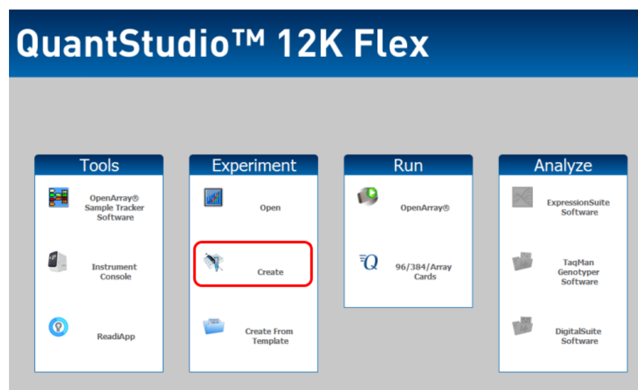
---

5. Click **Next**, and proceed to next step.



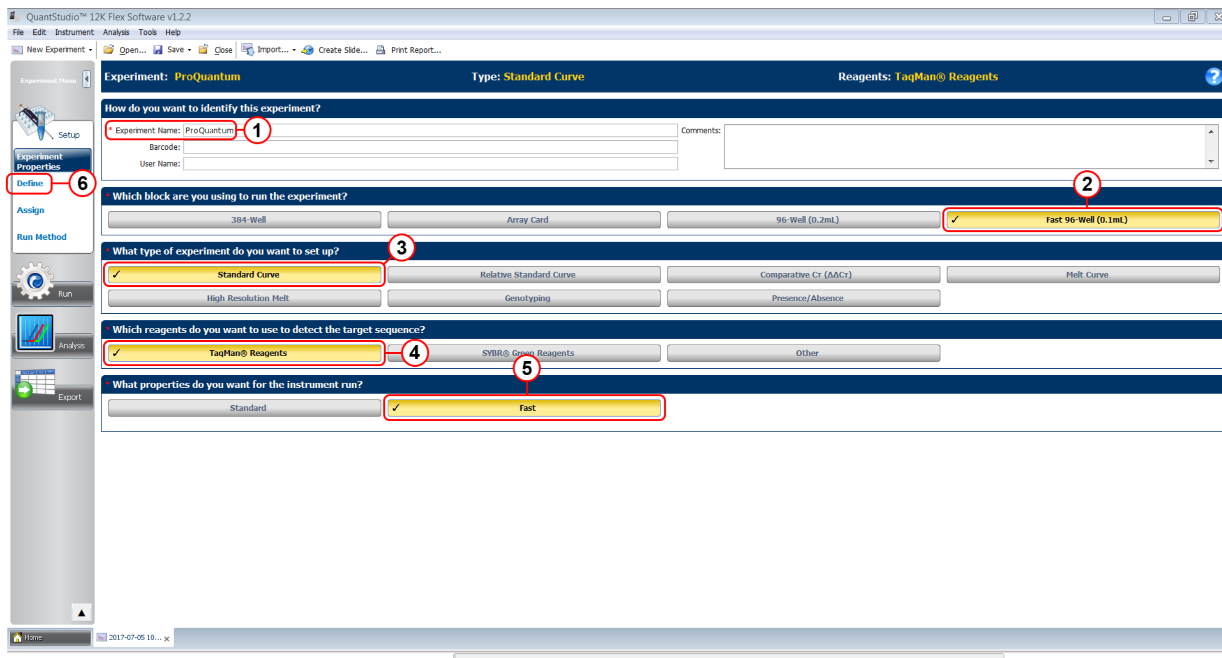
# Settings for the QS 12K System

Settings for the QuantStudio™ 12K Flex System can be modified from the **Create** screen.



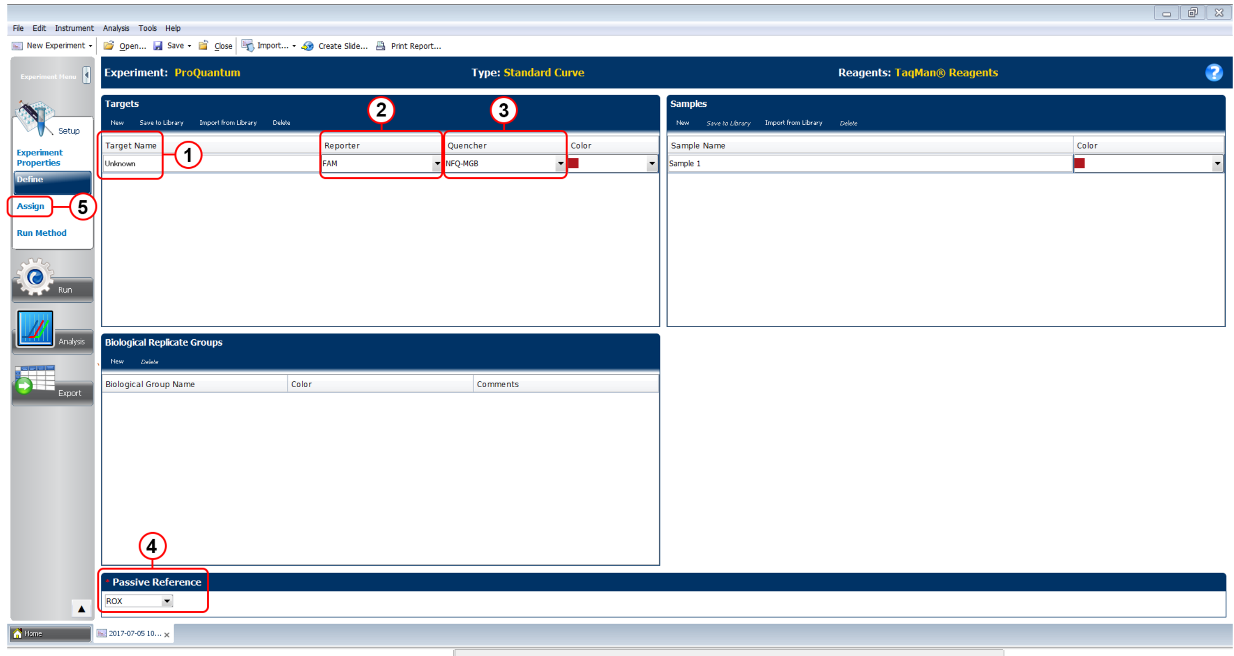

## Set up experiment properties

1. Enter "ProQuantum" as the **Experiment name**.
2. Select your block type (0.1 mL or 0.2 mL).
3. Ensure experiment type is set to **Standard curve**.
4. Ensure reagent type is set to **TaqMan™ Reagents**
5. Select the appropriate instrument properties based on your block type (**Fast** for 0.1 mL, or **Standard** for 0.2 mL).
6. Click **Define**, and proceed to next step.



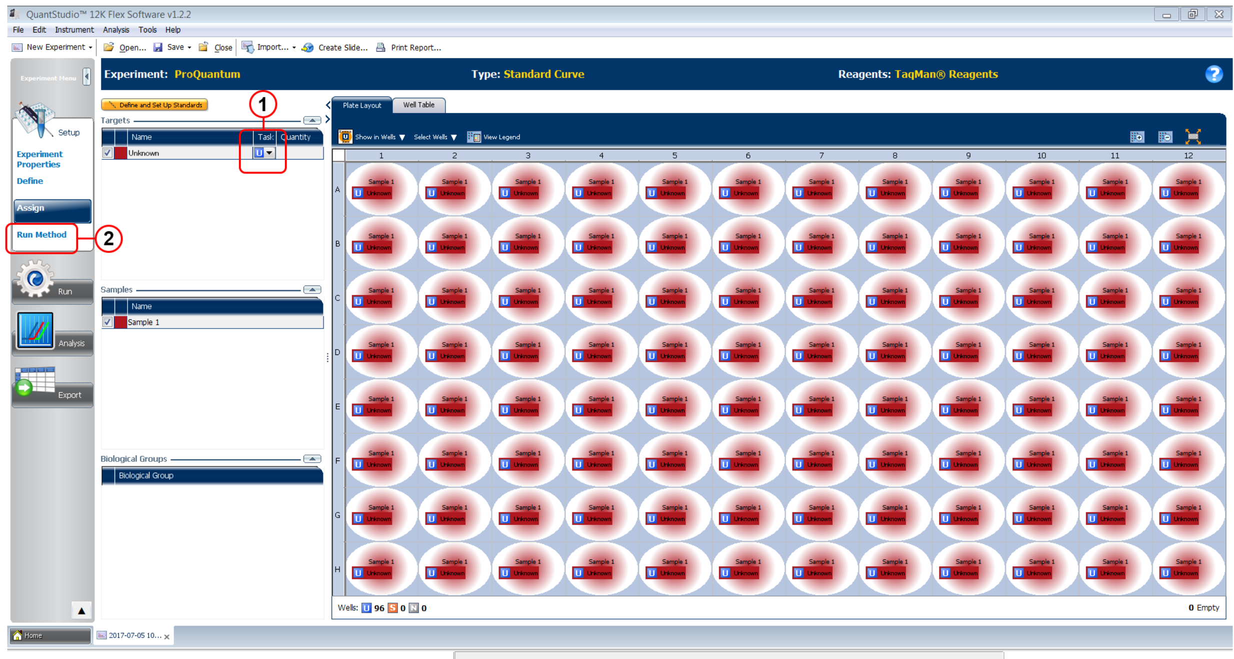

## Define target

1. Enter "Unknown" as the **Target name**.
2. Ensure the **Reporter** is set to **FAM**.
3. Ensure the **Quencher** is set to **NFQ-MGB**.
4. Ensure the **Passive reference** is set to **ROX**.
5. Click **Assign**, and proceed to next step.




## Assign wells

1. Assign all wells as unknown by setting **Task** to **U**.
2. Click **Run method**, and proceed to next step.



## Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

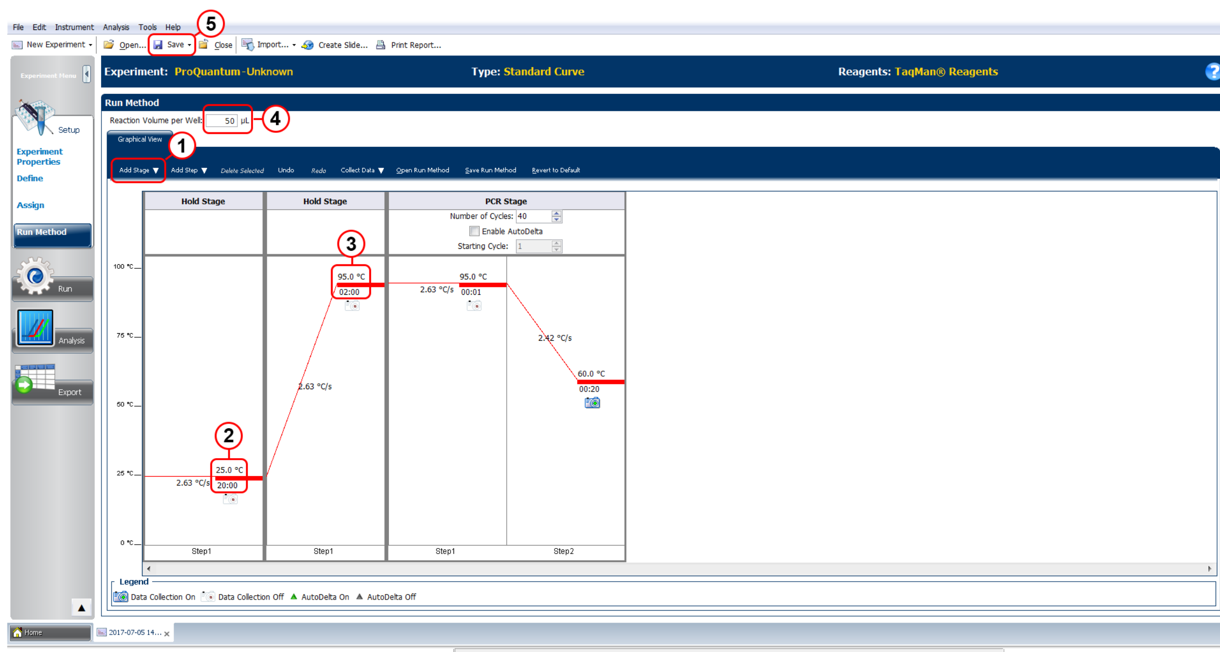
1. Add a new **Hold stage**.
2. Set first **Hold stage** to 25°C for 20:00.
3. Modify second **Hold stage** to 95°C for 2:00.
4. Set **Reaction volume per well** to 20 µL or 50 µL depending upon the protocol to be performed.

---

**Note:** The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio™ instruments only)" on page 17 for details.)

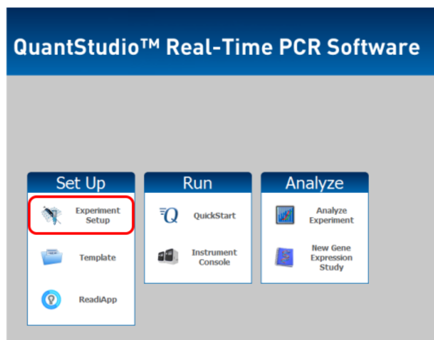
---

5. Save the Method.



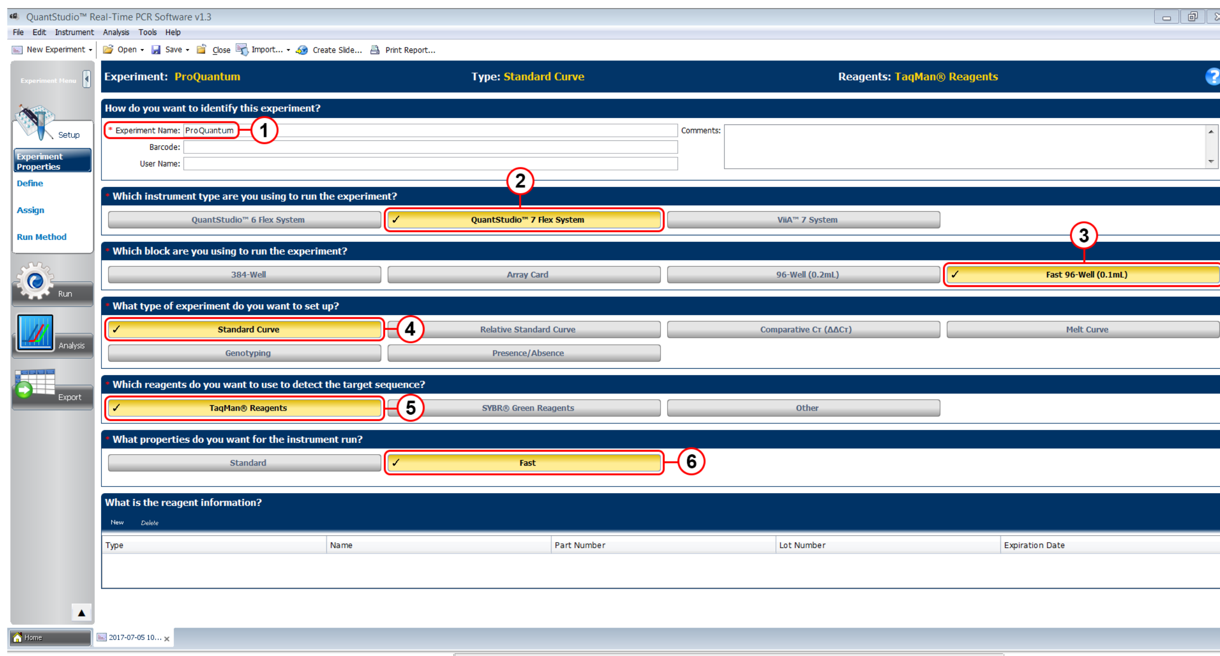
# Settings for QS 6 and 7 Systems

Settings for the QuantStudio™ 6 Flex System and QuantStudio™ 7 Flex System can be modified from the **Experiment setup** screen.



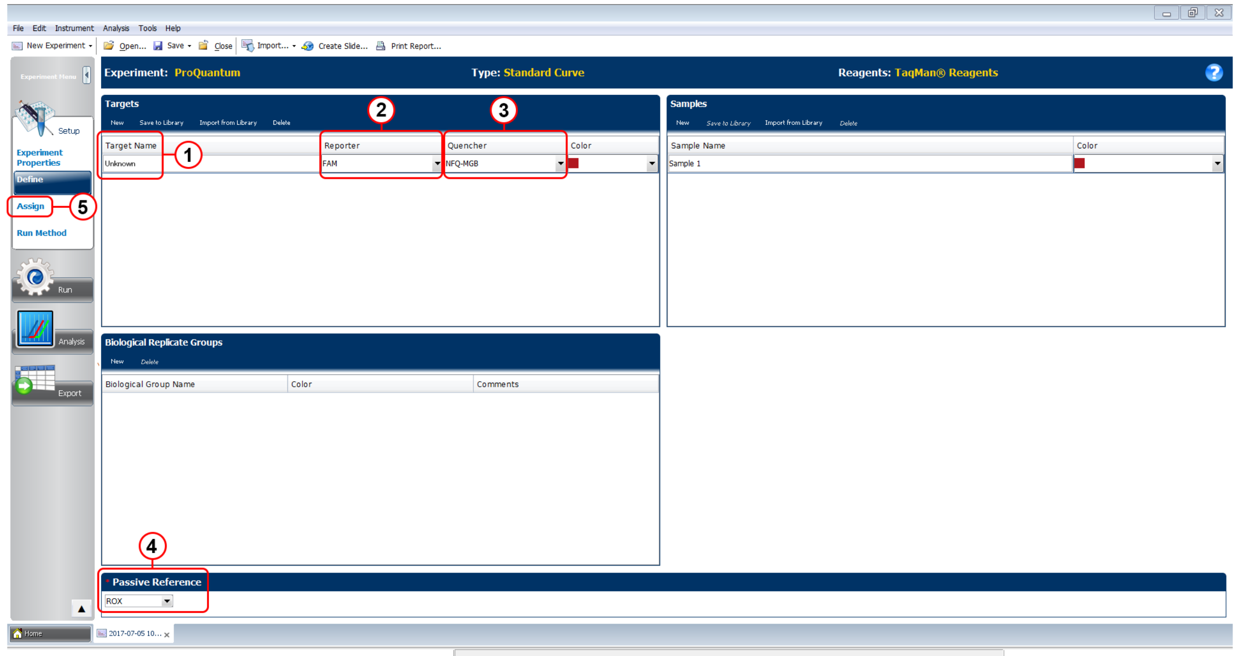

## Set up experiment properties

1. Enter "ProQuantum" as the **Experiment name**.
2. Select your instrument type (QuantStudio™ 6 Flex System, QuantStudio™ 7 Flex System, or ViiA™ 7 Real-Time PCR System).
3. Select your block type (0.1 mL or 0.2 mL).
4. Ensure experiment type is set to **Standard curve**.
5. Ensure reagent type is set to **TaqMan™ Reagents**
6. Select the appropriate instrument properties based on your block type (**Fast** for 0.1 mL, or **Standard** for 0.2 mL).
7. Click **Define**, and proceed to next step.



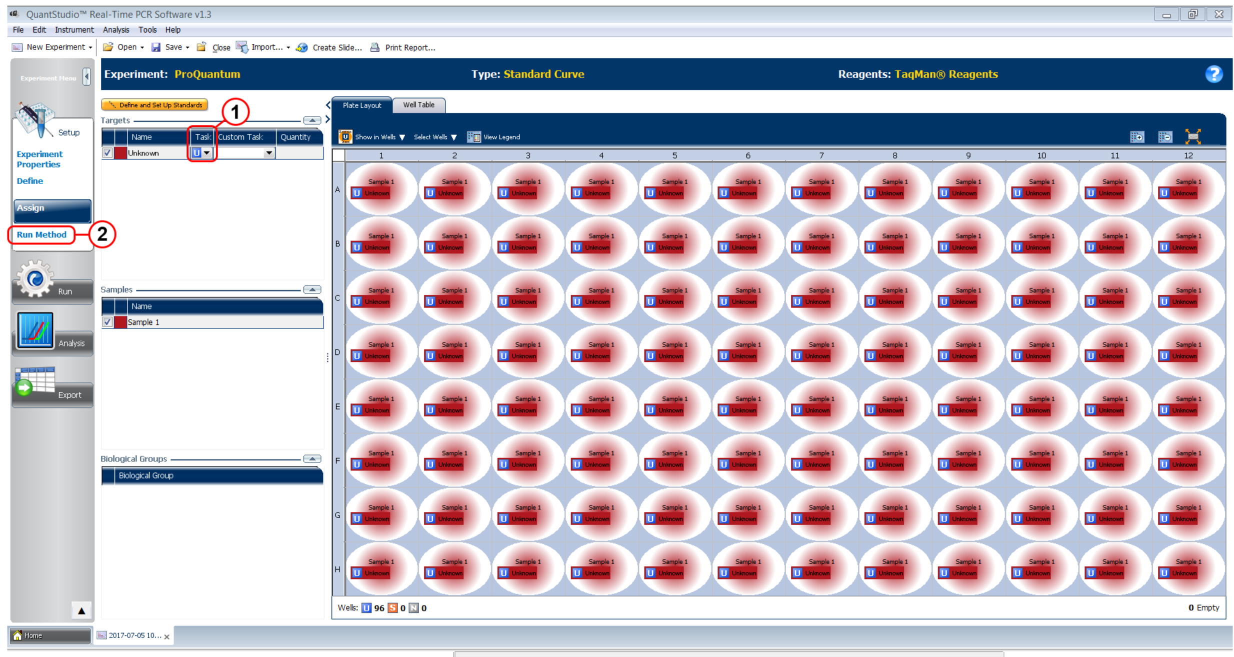

## Define target

1. Enter "Unknown" as the **Target name**.
2. Ensure the **Reporter** is set to **FAM**.
3. Ensure the **Quencher** is set to **NFQ-MGB**.
4. Ensure the **Passive reference** is set to **ROX**.
5. Click **Assign**, and proceed to next step.




## Assign wells

1. Assign all wells as unknown by setting **Task** to **U**.
2. Click **Run method**, and proceed to next step.



## Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

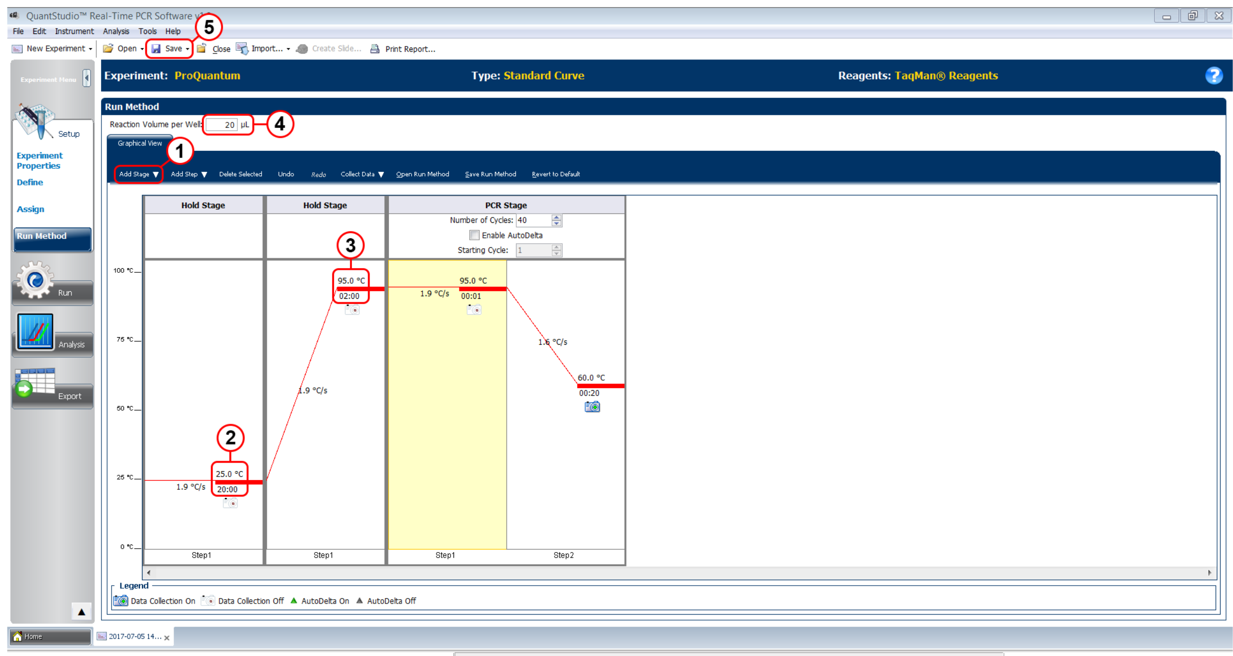
1. Add a new **Hold stage**.
2. Set first **Hold stage** to 25°C for 20:00.
3. Modify second **Hold stage** to 95°C for 2:00.
4. Set **Reaction volume per well** to 20 µL or 50 µL depending upon the protocol to be performed.

---

**Note:** The ramp rate must be modified if using a 50 µL reaction volume (see "Determine ramp rate (QuantStudio™ instruments only)" on page 17 for details.)

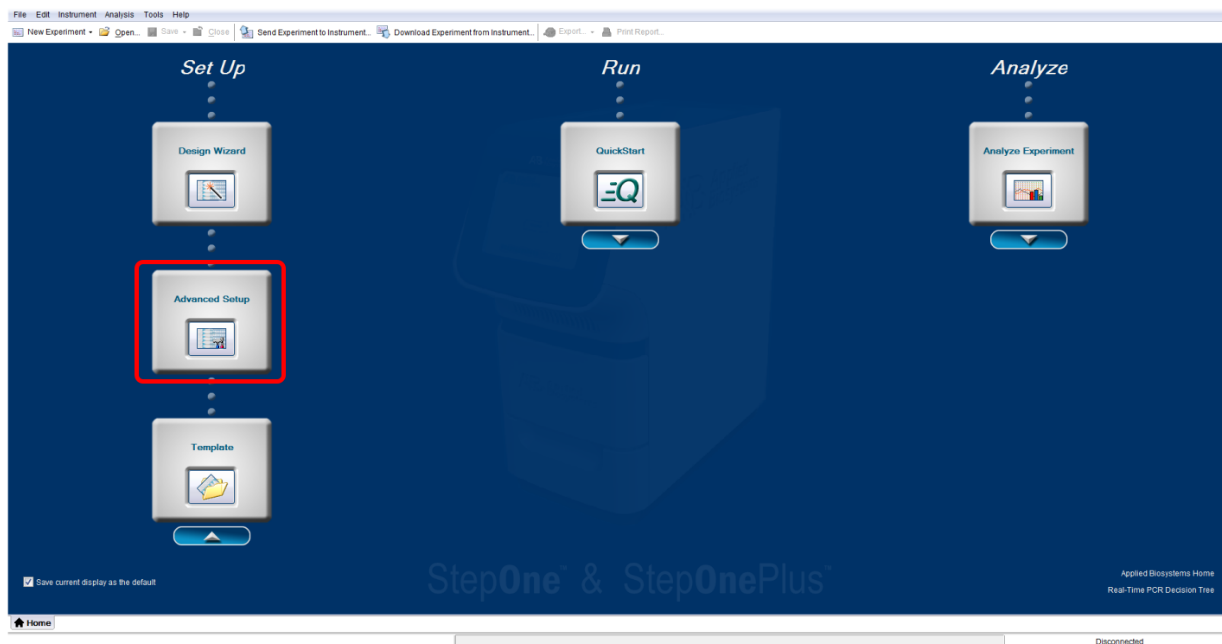
---

5. Save the Method.



# Settings for the StepOnePlus System

Settings for the StepOnePlus™ Real-Time PCR System can be modified from the **Advanced setup** screen.

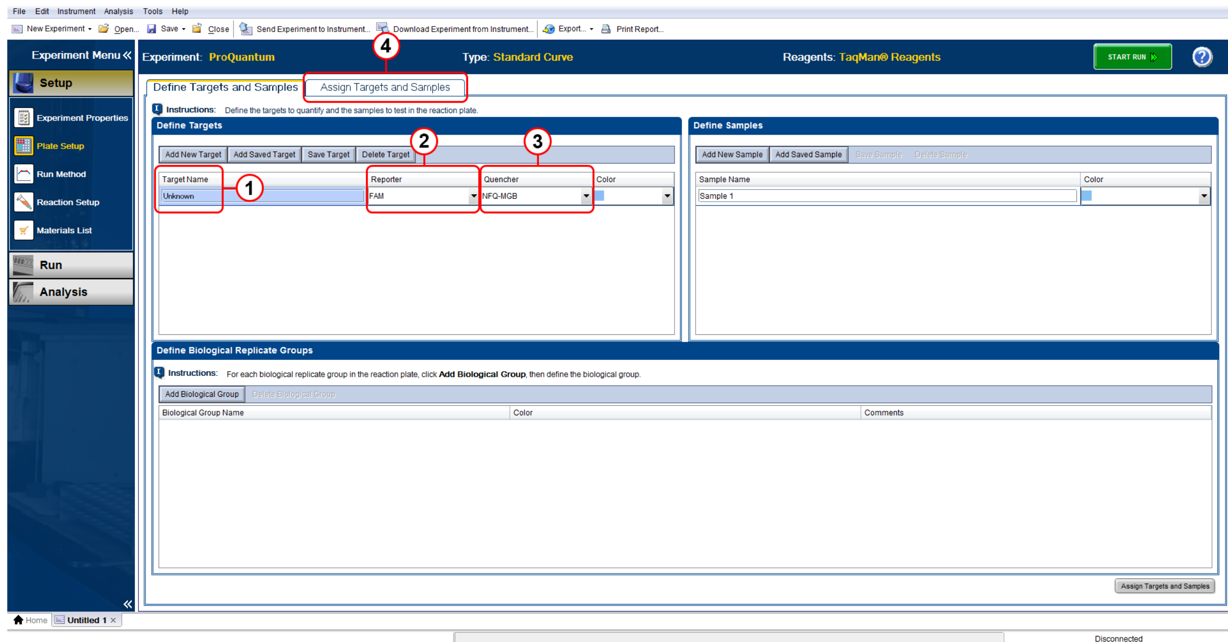


## Set up experiment properties

1. Enter "ProQuantum" as the **Experiment name**.
2. Select your instrument type (StepOnePlus™ Instrument (96 Wells or 48 Wells)).
3. Ensure experiment type is set to **Standard curve**.
4. Ensure reagent type is set to **TaqMan™ Reagents**
5. Select the appropriate instrument properties based on your block type (**Fast** for 0.1 mL, or **Standard** for 0.2 mL).
6. Click **Plate setup**, and proceed to next step.

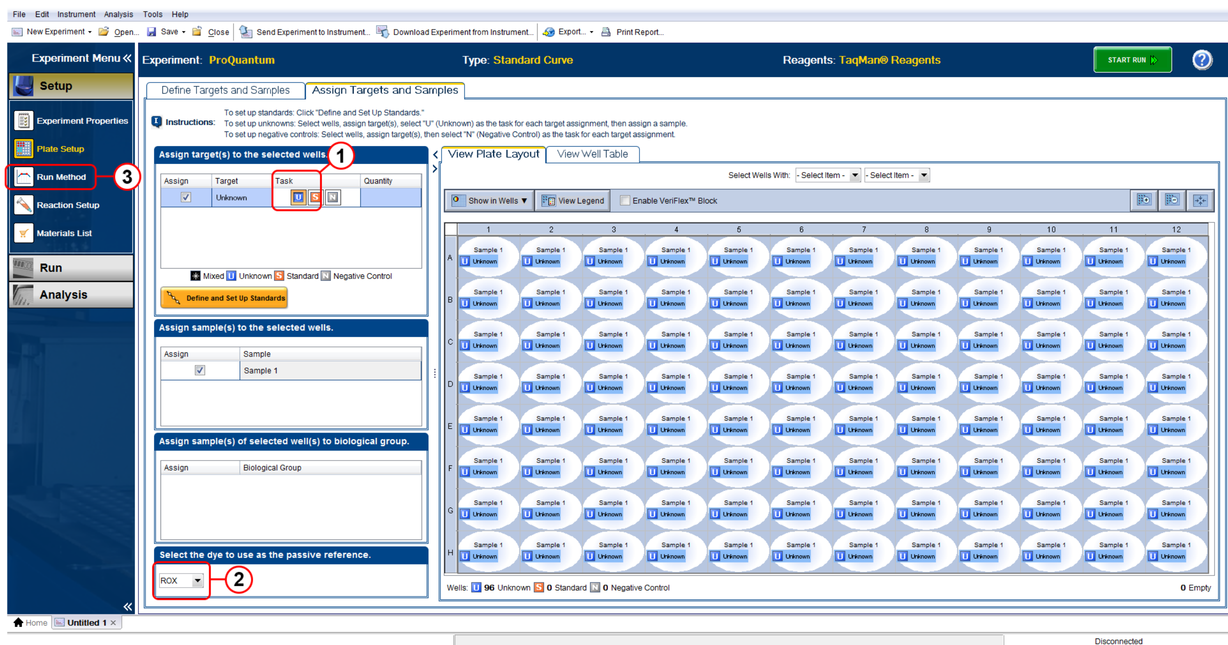
## Define targets and samples

1. Enter "Unknown" as the **Target name**.
2. Ensure the **Reporter** is set to **FAM**.
3. Ensure the **Quencher** is set to **NFQ-MGB**.
4. Click **Assign targets and samples**, and proceed to next step.




## Assign targets and samples

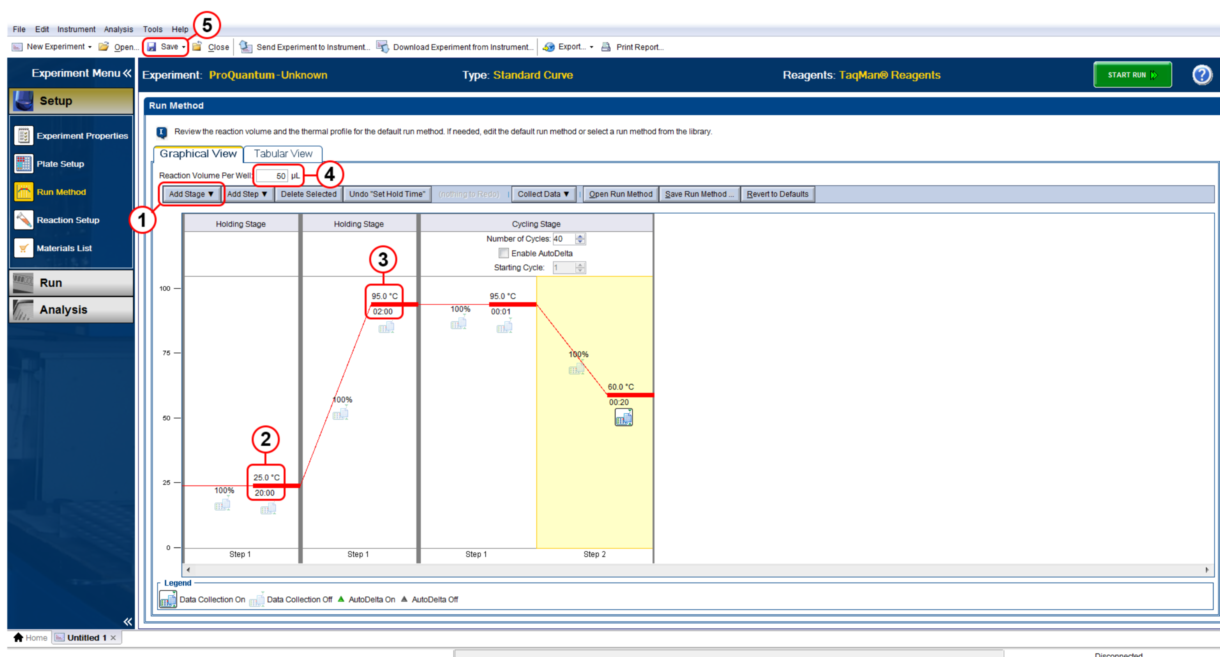
1. Assign all wells as unknown by setting **Task** to **U**.
2. Ensure the **Passive reference** is set to **ROX**.
3. Click **Run method**, and proceed to next step.



## Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

1. Add a new **Hold stage**.
2. Set first **Hold stage** to 25°C for 20:00.
3. Modify second **Hold stage** to 95°C for 2:00.
4. Set **Reaction volume per well** to 20 µL or 50 µL depending upon the protocol to be performed.
5. Save the Method.



## Importing .csv files

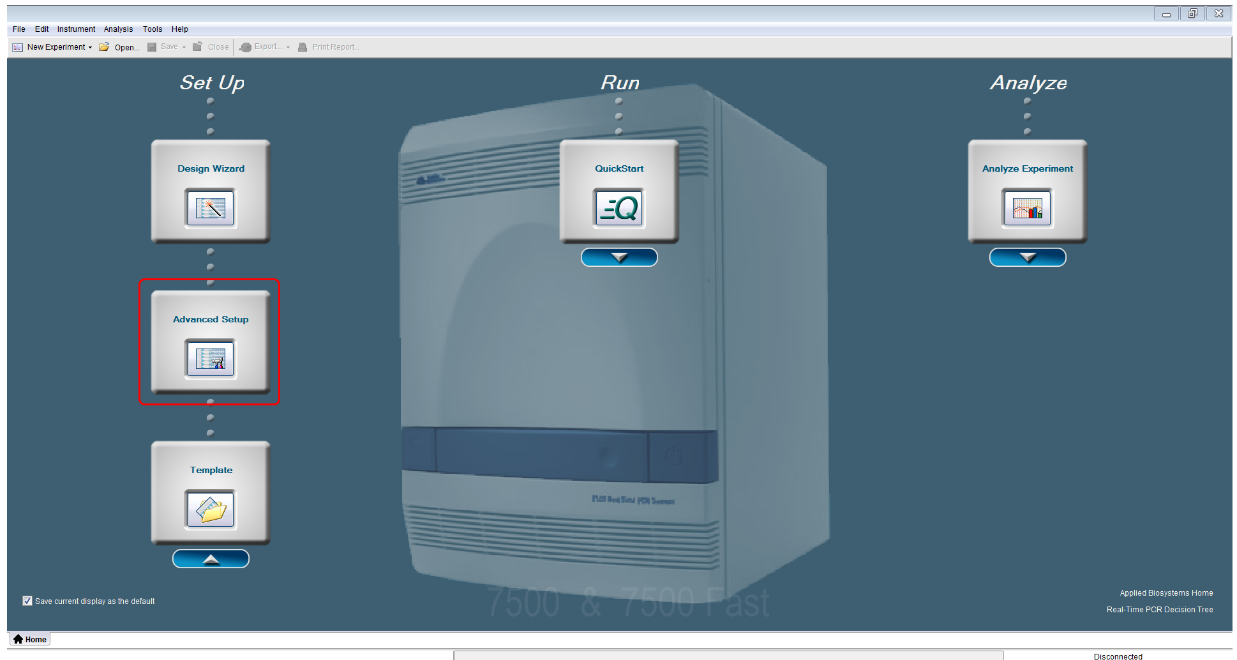
Data from qPCR instruments using 48-well blocks need to be imported as .csv files.

The .csv files need to have Ct data values formatted in rows and columns as represented on a 48-well plate as shown in the following layout. Do not include any headers with the .csv file.

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.417485 | 25.835571 | 17.634604 | 19.591108 | 26.777365 | 22.05344 | 24.465328 | 26.280548 |
| 2 | 25.531294 | 26.223822 | 26.246462 | 25.491188 | 25.321566 | 25.222649 | 26.042915 | 26.52998 |
| 3 | 25.489466 | 26.33618 | 26.282022 | 25.27978 | 26.006775 | 26.03464 | 26.18903 | 26.124218 |
| 4 | 25.222649 | 25.284784 | 25.775894 | 26.486937 | 25.348553 | 26.050512 | 26.934269 | 26.678078 |
| 5 | 15.477636 | 17.540136 | 25.805576 | 19.979145 | 22.435043 | 25.72326 | 24.326393 | 25.418428 |
| 6 | 27.274868 | 25.869804 | 26.293247 | 27.218275 | 26.051727 | 25.395878 | 25.971722 | 25.08545 |

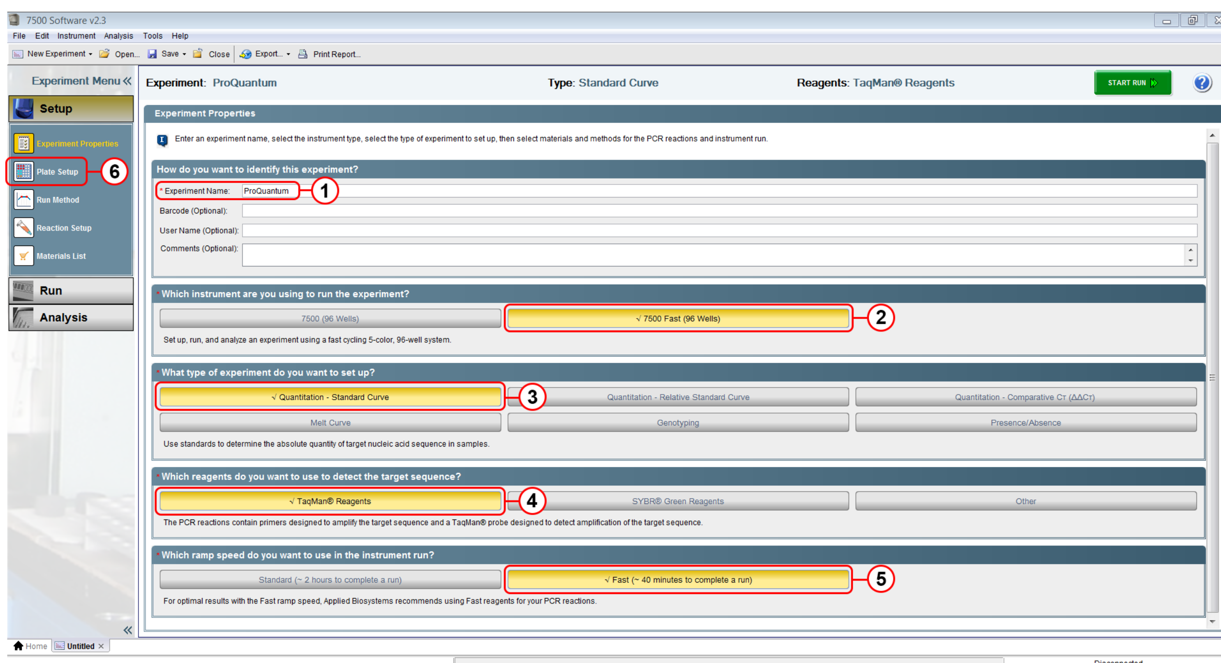
# Settings for the 7500 System

Settings for the 7500 Fast Real-Time PCR Instrument can be modified from the **Advanced setup** screen.



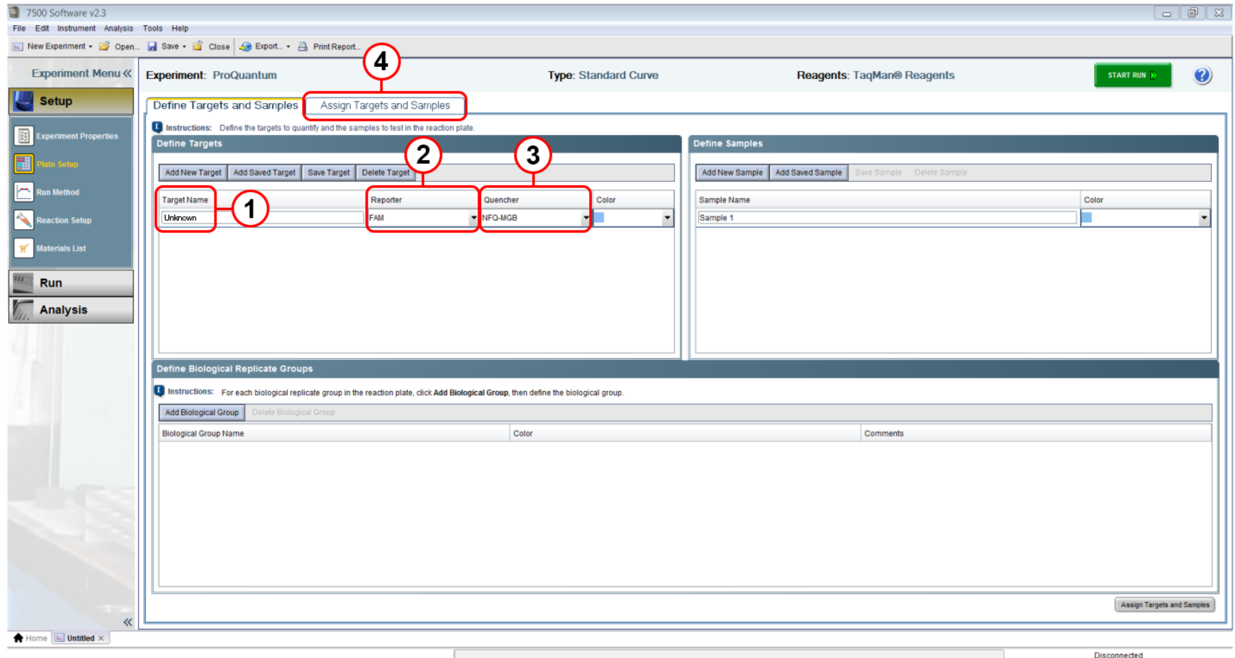
## Set up experiment properties

1. Enter "ProQuantum" as the **Experiment name**.
2. Select your instrument type (7500 or 7500 Fast).
3. Ensure experiment type is set to **Standard curve**.
4. Ensure reagent type is set to **TaqMan™ Reagents**
5. Select the appropriate instrument properties based on your block type (**Fast** for 0.1 mL, or **Standard** for 0.2 mL).
6. Click **Plate setup**, and proceed to next step.



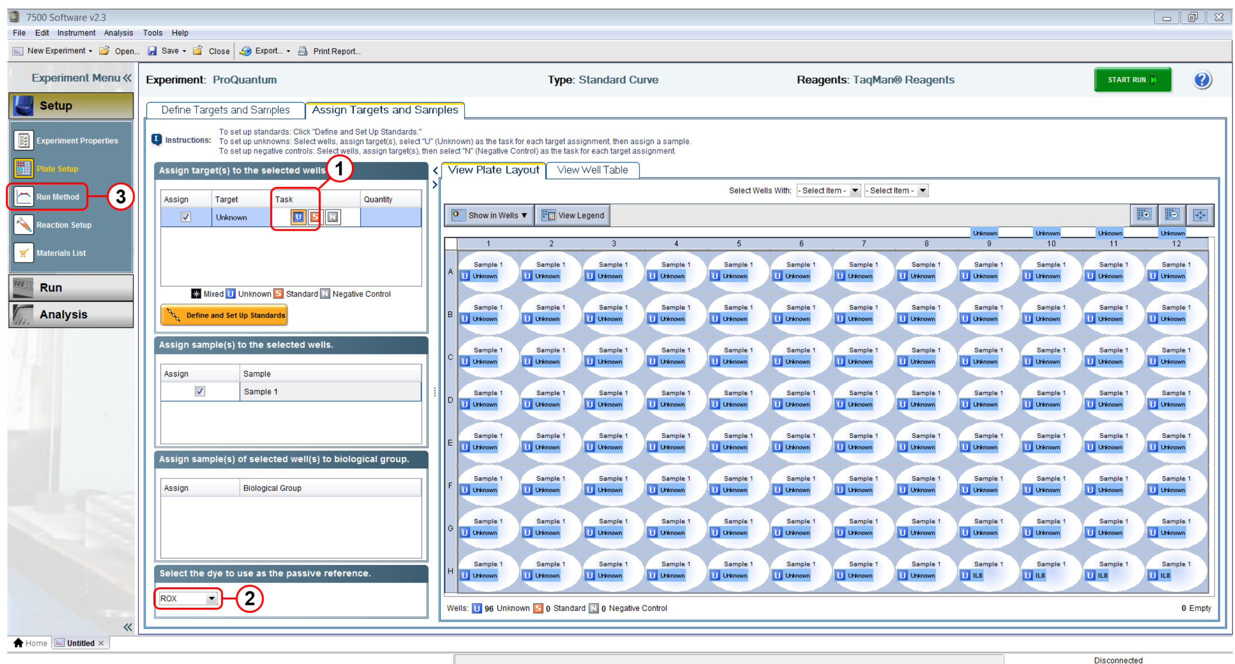

## Define targets and samples

1. Enter "Unknown" as the **Target name**.
2. Ensure the **Reporter** is set to **FAM**.
3. Ensure the **Quencher** is set to **NFQ-MGB**.
4. Click **Assign targets and samples**, and proceed to next step.




## Assign targets and samples

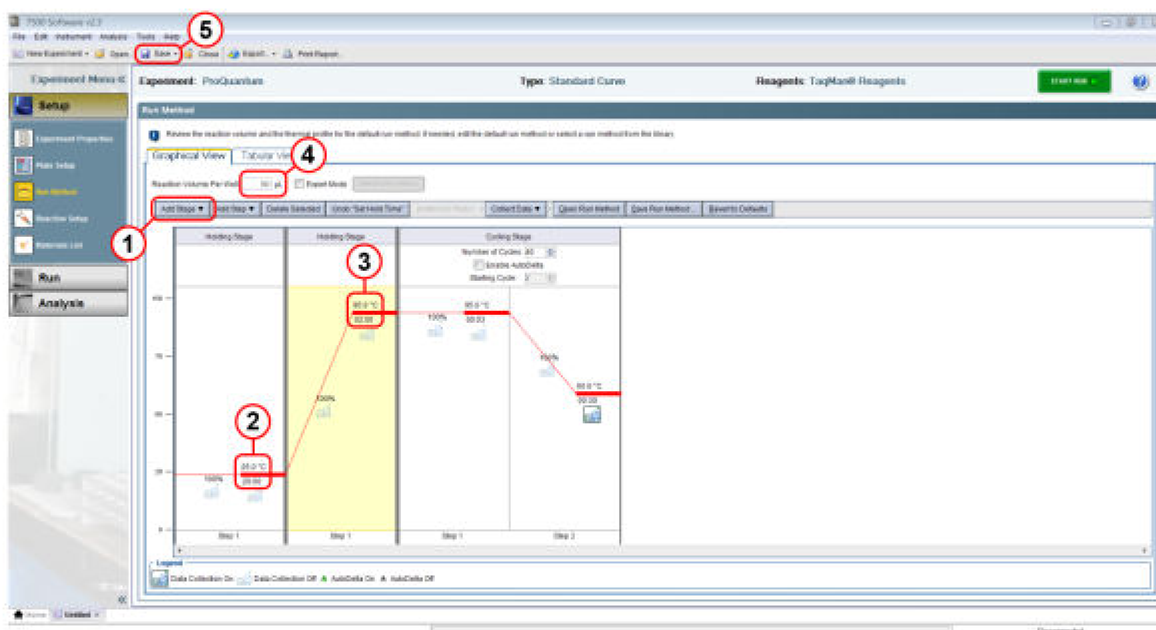
1. Assign all wells as unknown by setting **Task** to **U**.
2. Ensure the **Passive reference** is set to **ROX**.
3. Click **Run method**, and proceed to next step.



# Define Method

Except for adding a hold stage for ligation and modifying a hold stage for ligase inactivation, PCR conditions for the Method should use the default settings for the instrument.

1. Add a new **Hold stage**.
2. Set first **Hold stage** to 25°C for 20:00.
3. Modify second **Hold stage** to 95°C for 2:00.
4. Set **Reaction volume per well** to 20 µL or 50 µL depending upon the protocol to be performed.
5. Save the Method.



# Settings for non-AB instruments

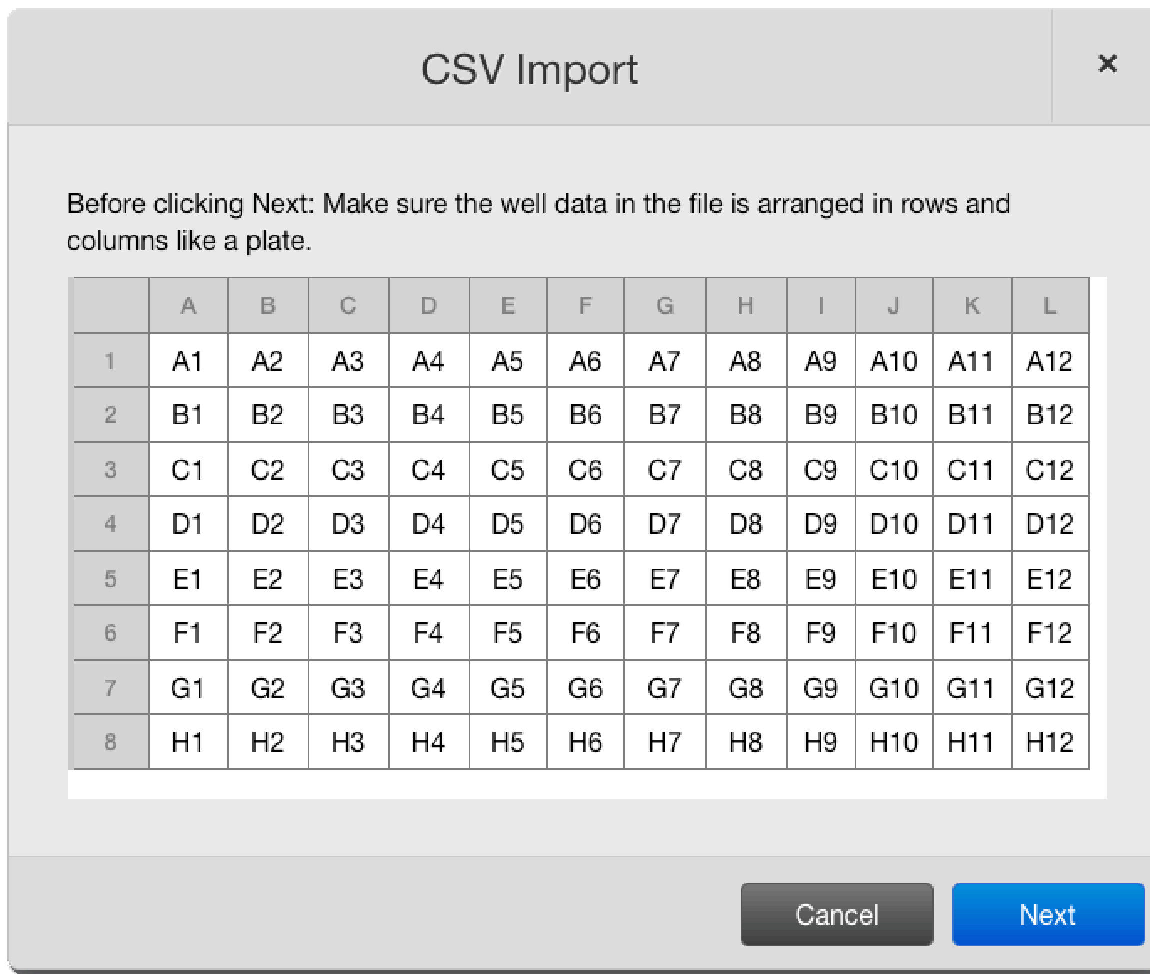
If you are using a qPCR instrument from another manufacturer, collect Ct values for each data point using the equivalent settings described in the following table.

| Parameter | Settings for Applied Biosystems™ instruments |
| --- | --- |
| Experiment type | Standard Curve or Quantitation - Standard Curve |
| Reagents | TaqMan™ reagents |
| Reporter dye | FAM |
| Quencher | NFQ-MGB [1] |
| Passive reference | ROX |
| Assign wells | Define all wells of the 96-well plate as Unknown |
| Threshold | 0.2 |
| Baseline | 3–15 |

[1] For instruments without this option, enter "None" or "Non-fluorescent".

## Importing .csv files

Data from qPCR instruments produced by other manufacturers need to be imported as .csv files.




| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| 2 | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| 3 | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| 4 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| 5 | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| 6 | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| 7 | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| 8 | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

The .csv files from non-Applied Biosystems instruments need to have Ct data values formatted in rows and columns as represented on a 96-well plate as shown in the following layout. Do not include any headers with the .csv file.

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 13.417485 | 25.835571 | 17.634604 | 19.591108 | 26.777365 | 22.05344 | 24.465328 | 26.280548 | 25.935783 | 27.044222 | 26.877268 | 26.834337 |
| 2 | 25.531294 | 26.223822 | 26.246462 | 25.491188 | 25.321566 | 25.222649 | 26.042915 | 26.52998 | 25.951874 | 29.698883 | 25.984228 | 25.489466 |
| 3 | 25.489466 | 26.33618 | 26.282022 | 25.27978 | 26.006775 | 26.03464 | 26.18903 | 26.124218 | 26.179691 | 25.951874 | 26.76191 | 26.373287 |
| 4 | 25.222649 | 25.284784 | 25.775894 | 26.486937 | 25.348553 | 26.050512 | 26.934269 | 26.678078 | 26.233883 | 29.54913 | 26.552887 | 26.263578 |
| 5 | 15.477636 | 17.540136 | 25.805576 | 19.979145 | 22.435043 | 25.72326 | 24.326393 | 25.418428 | 26.436768 | 27.017548 | 26.827032 | 26.700144 |
| 6 | 27.274868 | 25.869804 | 26.293247 | 27.218275 | 26.051727 | 25.395878 | 25.971722 | 25.08545 | 26.522614 | 3.4741602 | 26.413948 | 28.00413 |
| 7 | 25.90196 | 25.222649 | 26.21743 | 26.222464 | 25.189606 | 26.246462 | 26.463821 | 25.861534 | 25.971722 | 26.372057 | 25.813805 | 25.760149 |
| 8 | 25.333206 | 27.274868 | 25.303984 | 26.284954 | 27.218275 | 25.53206 | 25.848406 | 28.091894 | 25.992739 | 25.578075 | 27.65518 | 26.68899 |