

Spartan **DX-12**TM

Desktop DNA Analyzer

Quick Reference Guide

Version 3.10

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1. How to Use This Guide

Purpose of This Guide	This guide helps you prepare to receive and install the Spartan Bioscience DX-12™ DNA Analyzer System.
Audience	This guide is intended for personnel who will schedule, manage, and perform the tasks required to prepare your site for installation and operation of the Spartan DX-12™ system.
Assumptions	This guide assumes that you have: <ul style="list-style-type: none">• Familiarity with Microsoft Windows® XP and/or Microsoft Vista® operating system.• Familiarity with the Internet and Internet browsers.• Knowledge of techniques for handling DNA samples and preparing them for PCR.• An understanding of data storage, file transfer, copying and pasting.
Text Conventions	This guide uses the following conventions: <ul style="list-style-type: none">• Text in BOLD and quotation marks (""") indicates user action. For example: <i>Using the keypad, select the "Options" menu on the instrument.</i>• Bold, <i>Italic</i> & <u>underlined</u> text indicates new or important words and are also used for emphasis. For example: <i>This data can <u>ONLY</u> be graphed using Spartan Analyzer software that is supplied with the instrument.</i>

2. Instrument Installation

2.1. Unpacking

The Spartan DX-12™ is shipped in a cardboard container with molded inserts. The container should be carefully inspected for damage. Report any damage to your authorized Spartan Bioscience dealer before accepting the unit. We recommend you read the unpacking and setup instructions.

2.2. Instrument Contents

Spartan DX-12™ Desktop DNA Analyzer (Cat. No. 01004350)

12V Power Supply and Power Cord (Cat. No. 01004381)

USB Drive Containing All Supporting Materials (Cat. No. 01004429)

12 Centrifuge Adaptors (Cat. No. 01004247)

1 Setup Rack (Cat. No. 01004357)

Starter Pack of 100 Spartan tubes (20µl PCR Tube Assembly, Tubes - Cat. No. 01004153 and Caps - Cat. No. 01004155).



The Spartan DX-12™ should be used with manufacturer-supplied accessories only.

2.3. Operating Requirements

This instrument is designed for indoor use only.

Operate in ambient temperature between 20 and 25°C (59 and 77°F).

Do not operate in a cold room or a refrigerated area.

Operate in ambient relative humidity of 20 to 50%.

These specifications have been calculated for altitudes between 0 and 2,000 meters.

Ensure adequate front and rear ventilation.

2.4. Installation

1. Unpack and position the instrument on the workbench in the upright position. Allow 15 cm space in front and behind the instrument to ensure sufficient cooling of the electronic components.



Failure to provide ventilation space may impact the instrument's ability to maintain proper operating temperatures. This could negatively impact your reaction results and cause the instrument to overheat.

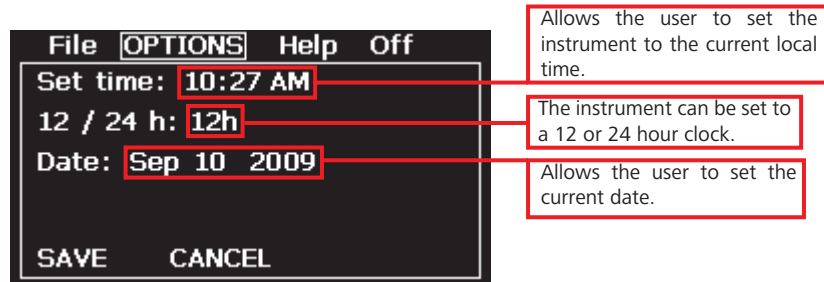
2. **Store original packaging in a safe location in case, at any time, the instrument should require shipping.**
3. Select a site for the instrument where:
 - Ambient temperature is maintained at 20 to 25°C. Avoid direct exposure to air conditioning, heating vents, and direct sunlight.
 - Relative humidity is 20 to 50%, non-condensing.

- Exposure to dust and drafts is minimal.
- There is minimal vibration to the instrument.
- An electrical outlet is available. The power supply automatically accommodates incoming voltages between 100-240 V AC and 50-60 Hz. Use of an un-interruptible power supply (UPS) is recommended.

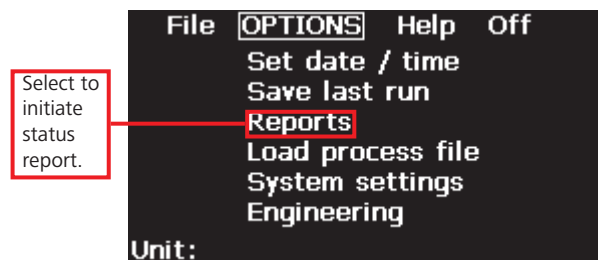


To prevent dust and debris from falling into the machine, keep the lid closed at all times.

4. Plug the power supply into the instrument and connect the power supply to power source.
5. The instrument is now operational. It will take 25-30 seconds for the screen to become active.



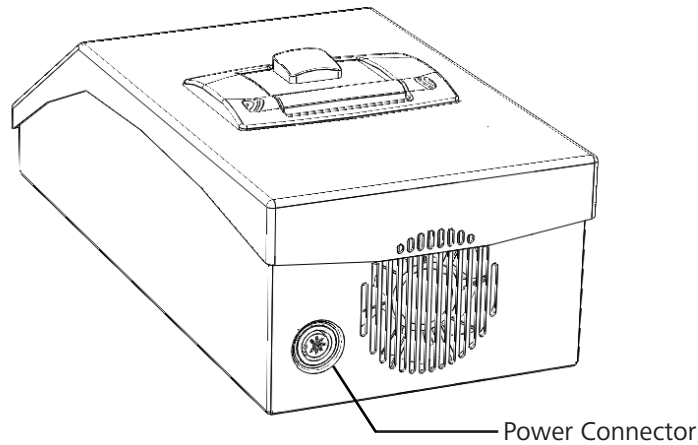
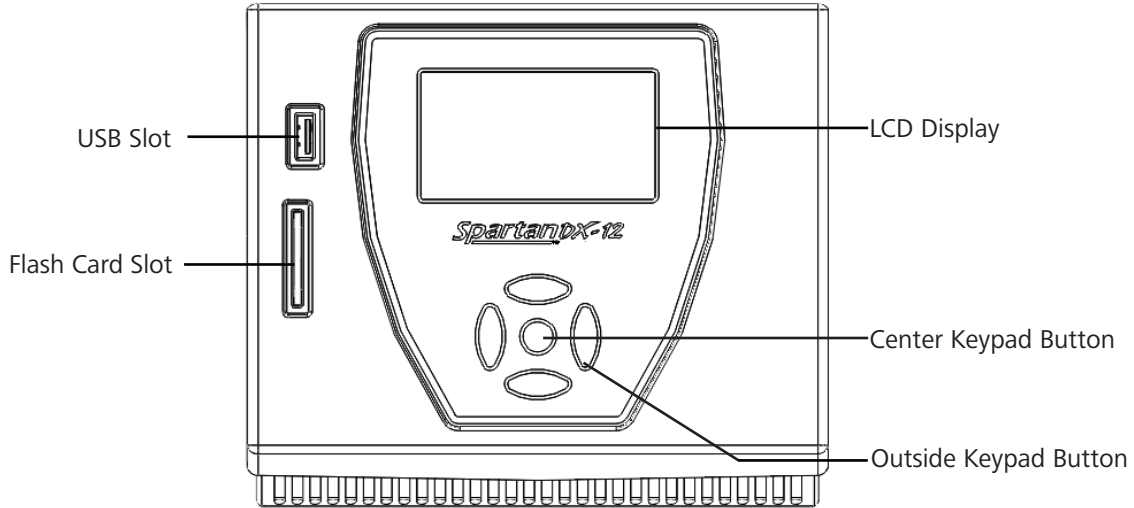
6. The first time you start the instrument the date and time will be displayed. We recommend you set the correct date and time, as all run data will be saved with this information. To do this:
 - Move to the appropriate **"Set Date/Time"** field using the outside buttons and select using the center button.
 - Modify the fields using outside buttons and unselect by pressing center button.
 - Select **"Save"** using the center button to save your new date and time settings.
7. Once the date and time have been set, we recommend the user initiate a status report, or instrument self test, to determine if the instrument has suffered any damage during the shipping process. Before initiating a status report, take a moment to transfer all the supporting documents from the USB stick to your personal computer (PC) for easy access.
8. Upon initial instrument running, we recommend you insert 12 empty tubes into the instrument, insert the provided USB key, navigate to the **"Options"** menu and initiate a report by selecting **"Reports"** from the menu. The report should take 10-20 minutes to run.



DO NOT remove the USB stick during this process. While the status report is running, it would be a good time to go through the additional supporting documentation provided on the USB, including the Spartan Graphing & Analysis Software. Once the status report has been completed, the instrument will return to the welcome menu, with all required

files saved to a folder named Status Report on the USB memory key. Please forward the results (3 files, named UUUUUreportDDMMYY.CSV, .PNG, or .RGB where UUUUU represents unit number, and DDMMYY represent the date) to support@spartanbio.com so that we may verify the functionality of the unit.

9. The instrument's lid should remain closed at ALL times except when tubes are being inserted or removed.



2.5. Decommissioning

The Spartan DX-12™ does not require any special action to remove it from use. However, as with any electronic equipment, please follow all applicable local, state/provincial, and/or national regulations for disposal.

3. Spartan Graphing & Analysis Software Installation

3.1. System Requirements

3.1.1. Hardware

800 MHz x 86 processor or higher

1024 x 768 display or higher

512 MB of RAM minimum

3.1.2. OS and Software

Windows® XP or higher

Software Installation

Spartan Graphing & Analysis software can be found on the USB memory key included with the Spartan DX-12™ instrument. To install the software, insert the USB memory key into your computer and run the installation program. The Spartan Graphing & Analysis software Setup Wizard will guide you through the steps required to install Spartan Graphing & Analysis software on your computer. Note that use of the software is intended for persons that either have licenses to perform PCR and real-time PCR, or are not required to obtain licenses. You must agree to the License Agreement in order to complete the installation.

To uninstall Spartan Graphing & Analysis software, run the installation program and select **“Remove Spartan Graphing & Analysis”**. Upgraded versions of software can be installed without the need to uninstall previous versions.

3.2. Troubleshooting/Support

For more information or help with Spartan Graphing & Analysis software, consult the Troubleshooting Guide (Doc. No. 01001097), or contact Spartan Bioscience by e-mail at support@spartanbio.com.

4. About Spartan Graphing & Analysis Software

The Spartan DX-12™ instrument produces run data in the form of data files, which are transferred between the device and a PC by USB memory key, or SD card. The run data is then imported and organized into three tabs: Run Data tab, Quantification tab, and Melt Curve tab.

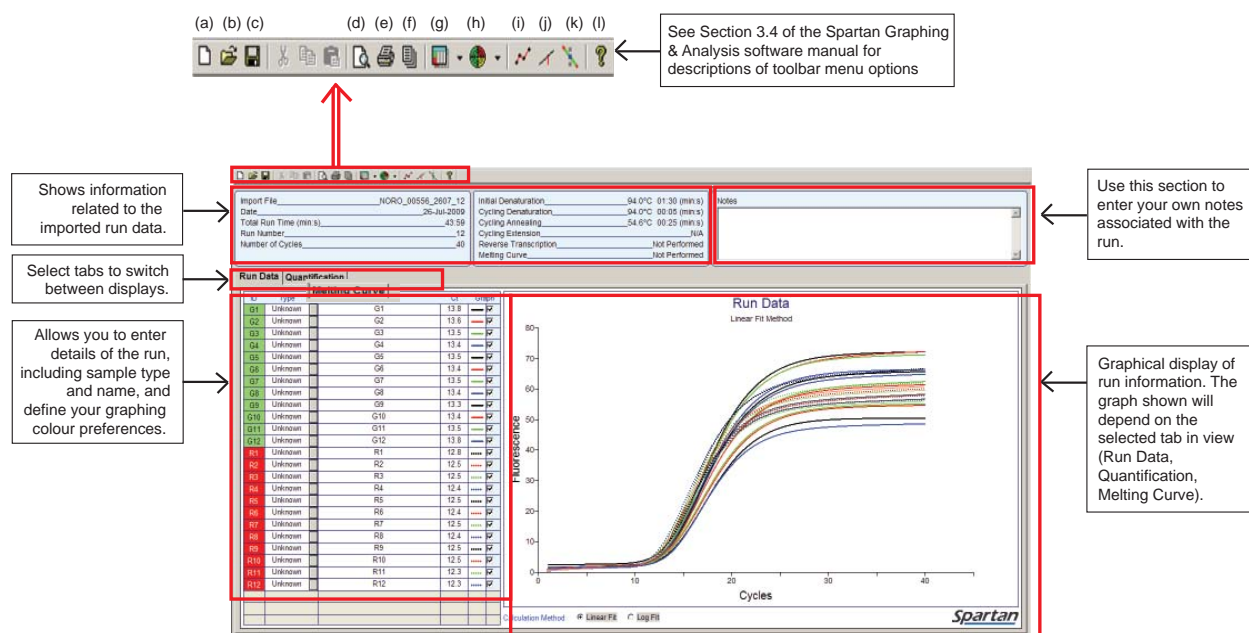



Figure 1. Default window display (Run Data tab) in Graphing & Analysis software.

 The view can be toggled between the three tabs (Run Data, Quantification, Melting Curve) by clicking with the mouse on the appropriate tab name (Figure 1). The Melting Curve tab only appears when melt analysis run data is present

There are two ways to load data into Spartan Graphing & Analysis software:


- A) **“Import Raw Data”** (Importing Data) - this option is used to load raw data generated from a run on the Spartan DX-12™ instrument into the graphing software. Files generated by the Spartan DX-12™ have the extension .txt. Once a .txt file has been imported into Spartan Graphing & Analysis software, the data can be saved as a .sdx file.
- B) **“Open a Data File”** (Opening a .sdx file) - this option is used to open a previously saved Spartan Graphing & Analysis file with the .sdx extension. Files with the extension .sdx can be reopened from within the software or can be opened directly, i.e. double-clicking on the file will launch the software and display the related data.

4.1. Importing Data

Results of Spartan DX-12™ runs are saved on the Flash card or USB memory key as non-editable encrypted data files, with the naming convention: AAAAAA_UUUUU_DDMM_RR.txt (where: AAAAAA is the name of the program ran on the Spartan DX-12™; UUUUU is the Spartan DX-12™ instrument number (can be found on the instrument welcome screen); DDMM is the day and month; RR is the run number of the day). Files are saved to the following location on the Flash card or USB memory key:

/rundata/UUUUU/DDMM_RR

UUUUU is the unit ID number, DDMM are day and month, and RR is run of the day. Encrypted data files can only be opened in Spartan Graphing & Analysis software. To import data into the software, use one of the following options:

- A) Select **"File"** then **"Import Raw Data"** from the toolbar menu; or
- B) Click on the Import icon  on the toolbar; or
- C) Use the Hotkey <Ctrl + I>.

The Import dialogue window will open (Figure 2). Navigate to the location of the saved data file and select **"Open"**. The data should now be loaded into the Spartan Graphing & Analysis software. The display will default to the Run Data tab (Figure 1).

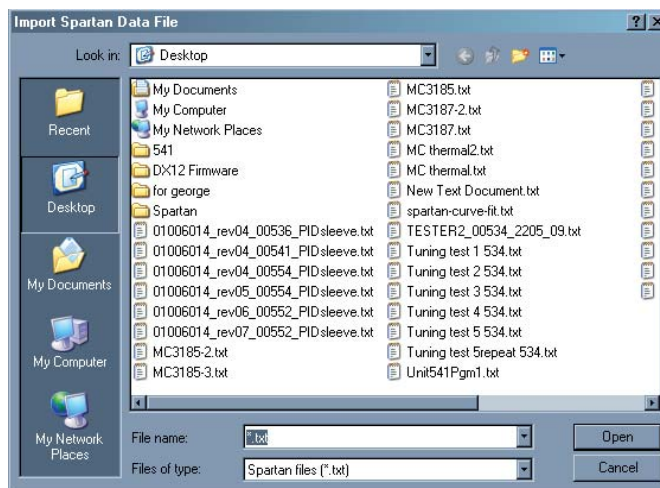



Figure 2. Import Data File dialogue window

4.2. Saving Data


Data imported into the software from a data file can be saved as a Spartan Graphing & Analysis file (.sdx extension), which can be reopened directly in the software. To save data as a .sdx file, use one of the following options:

- A) Select **"File"** then **"Save"** from the toolbar menu; or
- B) Click on the Save icon  on the toolbar; or
- C) Use the Hotkey <Ctrl + S>.

Once the data is saved as a .sdx data file, the name of the file will appear in the program title bar with the .sdx extension.

4.3. Opening an .sdx File

To open a previously saved .sdx file in Spartan Graphing & Analysis software, use one of the following options:

- A) Select **"File"** then **"Open a Data File"** from the toolbar menu; or
- B) Click on the Open icon  on the toolbar; or
- C) Use the Hotkey <Ctrl + O>.

For more detailed information on the functionality, or analysis specifics of the Spartan Graphing & analysis Software, please refer to the Spartan Graphing & Analysis User Manual (Doc. No. 01001044)

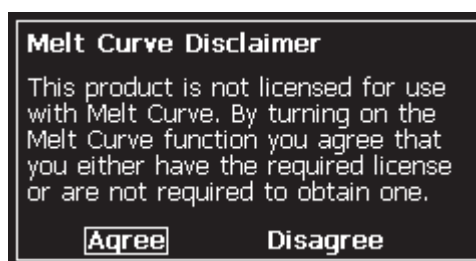
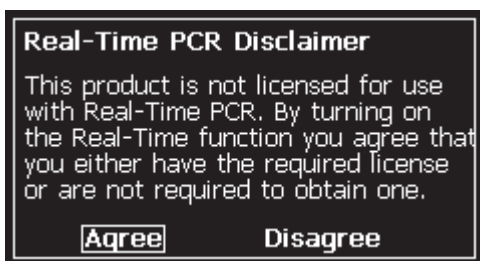
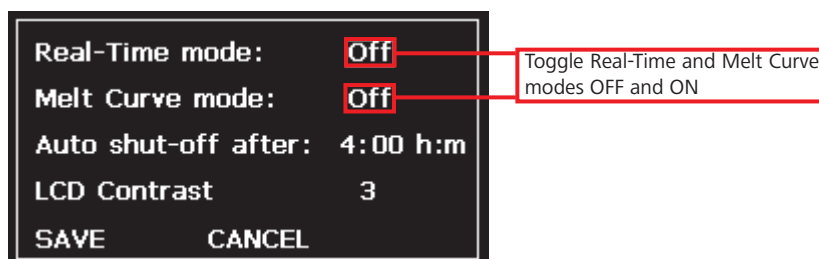
5. Operating Instructions

5.1. End-Point/Real-Time and Melt Curve Operational Modes

The Spartan DX-12™ is shipped in **End-Point** operational mode. In order to operate the unit in **Real-Time** or **Melt Curve** modes, these functions must be activated.

To activate **Real-Time** or **Melt Curve** modes:

1. Using the keypad, select the **“Options”** menu on the instrument.
2. Move to **“System Settings”** using the outside buttons and select using the center button.



3. Once within the **“System Settings”** menu, **“Melt Curve”** or **“Real-Time”** modes can be turned Off/On by moving to and selecting the appropriate field using the outside buttons and the center button, then toggling up and down with the outside arrow keys, pressing the center key again to register your selection.



The first time you change the Real-Time or Melt Curve operational setting, you will be prompted to “AGREE” or “DISAGREE” to a disclaimer. If you do not “AGREE” to the disclaimer, the Real-Time or Melt Curve modes will remain OFF, and will be inaccessible. Only once the disclaimers are “AGREED” to can you access these operational modes.

5.2. Reaction Set-up

5.2.1. Thermal Cycling

The Spartan DX-12™ is designed to run two- or three-temperature thermal cycling programs. A three-temperature program has three steps: denaturation, annealing, and extension. A two-temperature program combines the annealing and extension steps into a single step. Although three-temperature programs are more traditional, two-temperature programs can provide the same performance while significantly shortening run times.

Isothermal programs may also be performed on the Spartan DX-12™ by setting the denaturation, and annealing steps to the same temperature.

Note that the dwell time at each temperature begins once the reaction tube reaches the specified temperature. For most applications, only a few seconds are sufficient to denature the amplicon during thermal cycling.

5.3. Designing a Two-temperature Program

Each gene target is unique and may require optimization of the cycling program. Here are some general guidelines for determining the appropriate temperatures and dwell times for your program:

1. Design primers with matching melting temperatures (T_m), preferably between 60-68°C, and amplicon size of < 300 base pairs (bp). For faster cycling speeds, the primers should have T_m s of 65-68°C, and amplicon size of < 150 bp.
2. Set the annealing/extension temperature to the calculated T_m .
3. Set the annealing/extension time to 30 seconds.
4. For denaturation temperatures and times, we recommend starting with the following settings:

DNA Source	Denaturation Temp	Initial Time	Cycling Time
Human/Mammalian	95°C	2.5 min	10 s
Bacterial/Viral	95°C	1 min	10 s

5.3.1. Detection Chemistries

The Spartan DX-12™ is a 2-channel instrument. The first channel detects green wavelengths (520-532 nm), and the second channel detects red wavelengths (620-800 nm). The detection system is compatible with standard probe chemistries such as TaqMan® probes, Scorpion probes and Molecular Beacons. It is also compatible with DNA intercalating dyes such as SYBR Green® (Life Technologies). For probe technologies, Spartan recommends the use of non-fluorescent quenchers such as BHQ-1 and BHQ-2.

For SYBR Green I dye (Invitrogen, Cat. No. S7563), we recommend a concentration of 0.5X. However concentrations in the range of 0.2-1.0X are acceptable.

5.3.2. Reaction Tubes

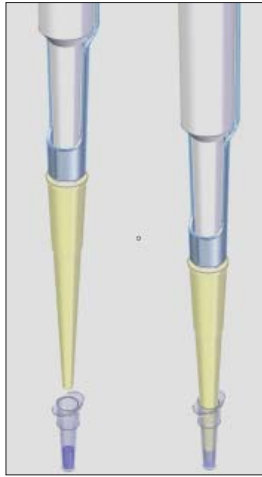
The Spartan DX-12™ is designed to work with Spartan reaction tubes (20µl PCR Tube Assembly, Tubes - Cat. No. 01004153 and Caps - Cat. No. 01004155). These tubes have been optimized for fast heating and cooling kinetics, and must be used to perform PCR on the Spartan DX-12.

Spartan reaction tubes are designed to be loaded directly with a pipette tip. To load tubes:

1. Insert loaded pipette tip to the bottom of the tube.
2. Slowly release the liquid while raising the pipette tip.



This should ensure that no bubbles are created and that the liquid does not go up the sides of the tube, as this will affect the proper seal of the cap.



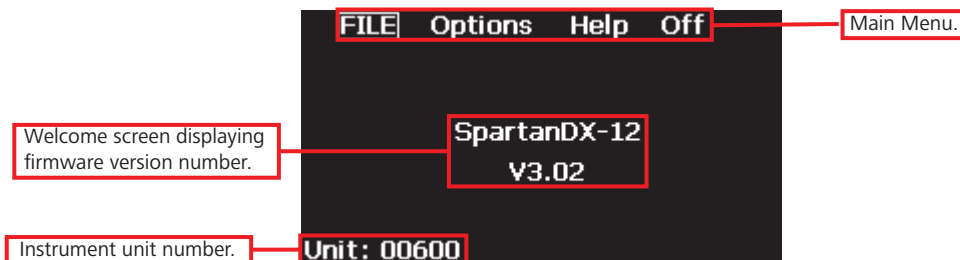
5.3.3. Reaction Volumes

Spartan reaction tubes have a maximum void volume of 25 μl . The minimum reaction volume is 10 μl . Spartan recommends reaction volumes of 20 μl .

6. Run Program

6.1. Warming Up and Turning Off

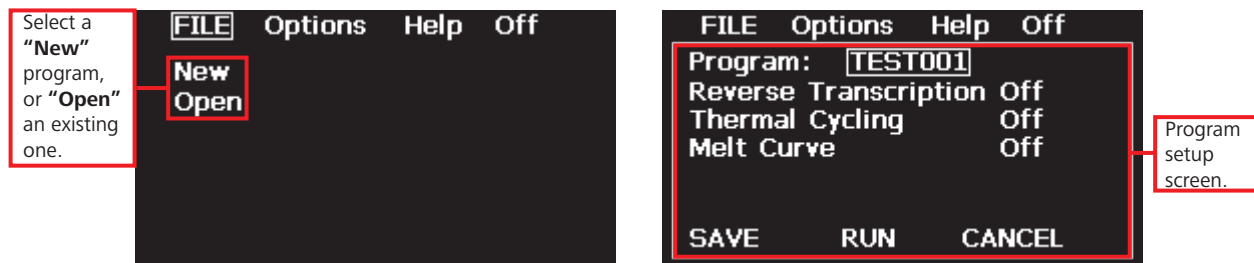
1. Plug in the Spartan DX-12™.
2. There is no power switch on the Spartan DX-12™. The welcome screen will appear automatically 25-30 seconds after plug-in:



3. Once the instrument is turned on, and you enter into the setup window, the instrument requires 5 minutes to warm up and equilibrate to room temperature.
4. To manually turn off the instrument, select **“Off”** from the Main Menu. When the instrument is turned **“Off”** all features of the instrument including fans and heaters are turned off. However, the instrument is effectively in **sleep mode** and can be turned back on by pressing any button.
5. To ensure that there is no power going to the instrument, unplug the power cord.

6.2. Selecting a Program

1. Use the left and right outside keypad buttons on the keypad to move to the **“File”** menu. Press the center button to select this menu.



2. To create a **New Program**, use the outside keypad buttons to navigate down to the **“New”** menu and select it. In the program setup screen, edit the name of your new program by pressing the center button. Use the up and down outside keypad buttons to adjust the characters, and use the left and right outside keypad buttons to move from one character to the next. When you are finished, press the center keypad button to accept the new program name.
3. To open an **Existing Program**, use the outside keypad buttons to navigate down to the **“Open”** menu and select it. Use the up and down buttons to move between programs in the program list. Programs will be displayed in numeric, then alphabetical order. Select a program by pressing the center button. Open the program by selecting **“Open”** from the options on the bottom of the screen. At any time, you may select a program

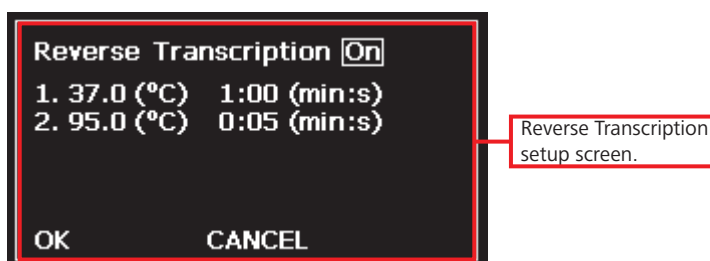
using the center button, and use the outside keypad buttons to move between the **“Open”**, **“Delete”**, and **“Cancel”** options at the bottom of the screen.

6.3. Setting Up a Program

1. After you have created a new program, or have opened an existing program, the program setup screen allows you to specify settings for the **Reverse Transcription**, **Thermal Cycling**, and **Melt Curve** steps in your program.
2. Use the outside keypad buttons to navigate between the program steps. Select **“On”** or **“Off”** to toggle the appropriate steps. Note that the instrument will perform the steps in the following order: Reverse Transcription → Thermal Cycling → Melt Curve. Steps set to **“Off”** will not be performed.
3. To save modifications to your program, select the **“Save”** option at the bottom of the screen. If you modify any of the three components of a program and run it without saving, the program will run as entered, but the changes will be lost at the end of the run.

6.3.1. Reverse Transcription

1. To modify the Reverse Transcription temperatures and dwell times, use the outside keypad buttons to navigate to the word **“Reverse Transcription”** and select it using the center button.

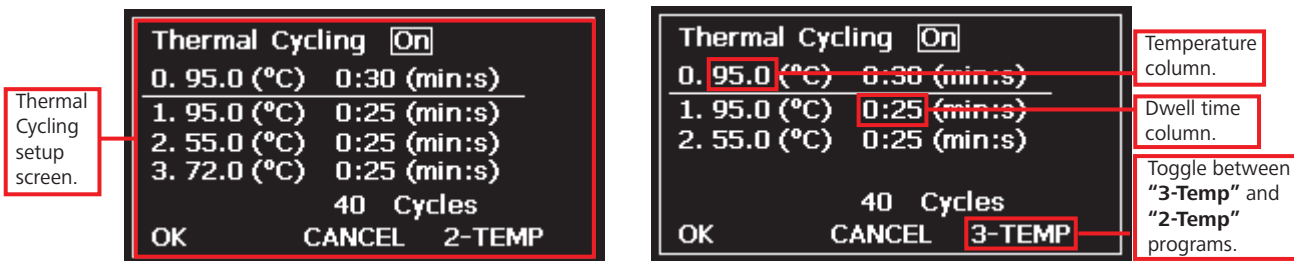


2. In the Reverse Transcription setup screen, use the outside keypad buttons to navigate between the temperatures and dwell times. After using the center button to select a setting to change, use the up and down outside keypad buttons to change the values. Use the center button to accept the new entry.
3. Step #1 specifies the Reverse Transcription synthesis conditions, and Step #2 specifies the Reverse Transcription denaturation conditions. Step #2 of the Reverse Transcription program, and Step #0 of the Thermal Cycling program are performed sequentially when both programs are **“On”**. This should be kept in mind when setting up a program that involves a Reverse Transcription step.
4. When you are finished adjusting the values, navigate to the bottom of the screen and select **“OK”**.
5. To cancel your modifications, navigate to the bottom of the screen and select **“Cancel”**.

6.3.2. Thermal Cycling

1. To modify the Thermal Cycling temperatures and dwell times, use the outside keypad buttons to navigate to the word **“Thermal Cycling”** and select it using the center

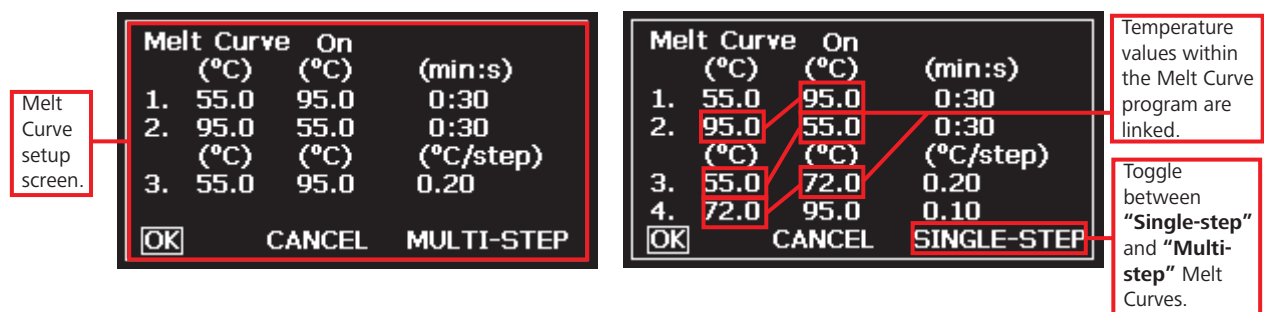
button.



- In the Thermal Cycling setup screen, use the outside keypad buttons to navigate between the temperatures, dwell times, and cycle number. After selecting a setting to change, use the up and down outside keypad buttons to change the values. Use the center button to accept the new entry.
- Step #0 specifies the initial denaturation conditions. During the cycling phase of the program, Steps #1, 2, and 3 specify the denaturation, annealing, and extension parameters, respectively.
- When you are finished adjusting the values, navigate to the bottom of the screen and select "OK" by pressing the center button.
- To cancel your modifications, navigate to the bottom of the screen and select "Cancel".
- By default Thermal Cycling is set to "3-Temp" thermal cycling conditions. To switch between two-temperature and three-temperature programs, toggle the "2-Temp" or "3-Temp" option at the bottom of the screen by selecting it with the center button.

6.3.3. Melt Curve

- To modify the Melt Curve temperatures and dwell times, use the outside keypad buttons to navigate to the word "Melt Curve" and select it using the center button.

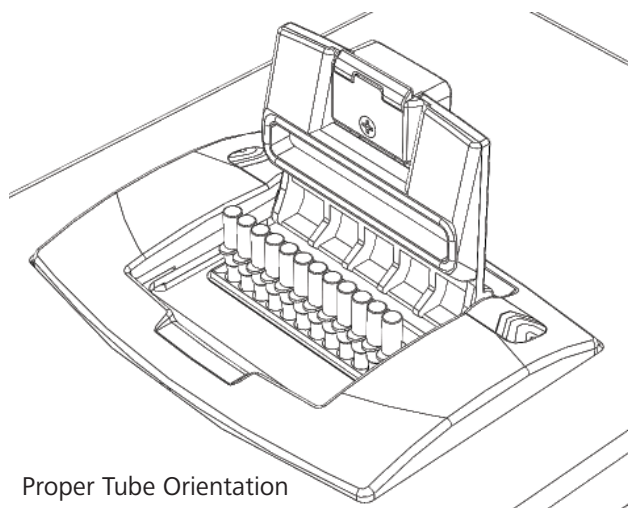


- In the Melt Curve setup screen, use the outside keypad buttons to navigate between the temperatures and dwell times. After selecting a setting to change with the center keypad button, use the up and down outside keypad buttons to change the values.
- Step #1 specifies the initial denaturation conditions. Step #2 specifies the starting temperature for the melt curve. Step #3 specifies the end temperature for a single-step melt curve. Step #4 specifies the end temperature for a multi-step melt curve. "°C/step" specifies the frequency of data acquisition. The minimum and maximum increments are 0.1°C/step and 1.0°C/step, respectively.

4. When you are finished adjusting the values, navigate to the bottom of the screen and select **"OK"**.
5. To cancel your modifications, navigate to the bottom of the screen and select **"Cancel"**.
6. By default, the Melt Curve program is set to **"Single-Step"**. A Single-Step program uses the same temperature increment from the starting to end temperatures. To switch between single-step and multi-step programs, toggle the **"Multi-Step"** to **"Single-Step"** option at the bottom of the screen by selecting it. A Multi-Step program allows you to set two different temperature increments between the starting and end temperatures. This feature is useful if you want higher resolution for a specific region of the curve, but want the overall run-time to be short.

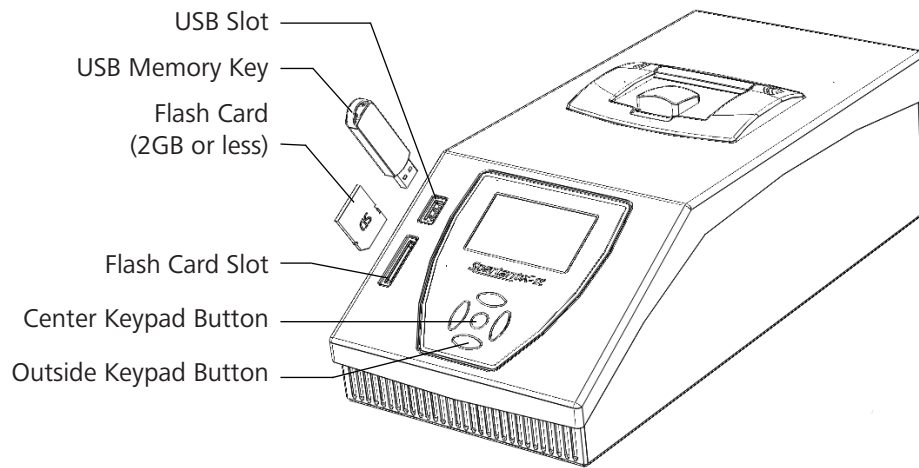
6.4. Running a Program

1. Insert Spartan reaction tubes into the instrument. Ensure that the tubes are properly nested in each well. **Do not force the tubes down into the wells.** Close the instrument lid. The lid will apply even pressure to ensure uniform heating.



Proper Tube Orientation

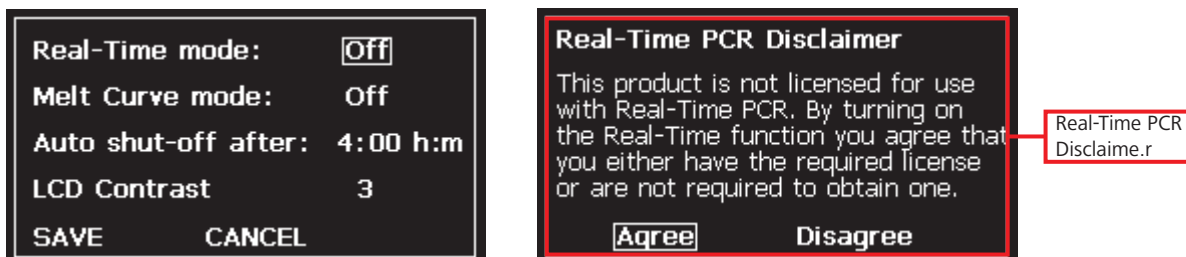
2. From the program setup screen, select the **"Run"** option from the bottom of the screen.
3. While the program is running, you may increase the cycle number at any time by navigating to the cycle number denominator on the screen with the outside keypad, pressing the center button to select it, and the up and down arrow key to make the changes, pressing the center button again to implement the changes.
4. While the program is running, you may also select **"Stop"** at any time. Note that the program is only stopped after you select **"Yes"** to confirm stopping the program. When a program is stopped, all data accumulated to that point has been saved and is available via the **"Save Last Run"** feature in the **"Options"** menu.
5. If a program is completed, and the instrument goes into the **Auto Off** mode, data can be saved through the **"Save Last Run"** feature in the **"Options"** menu.



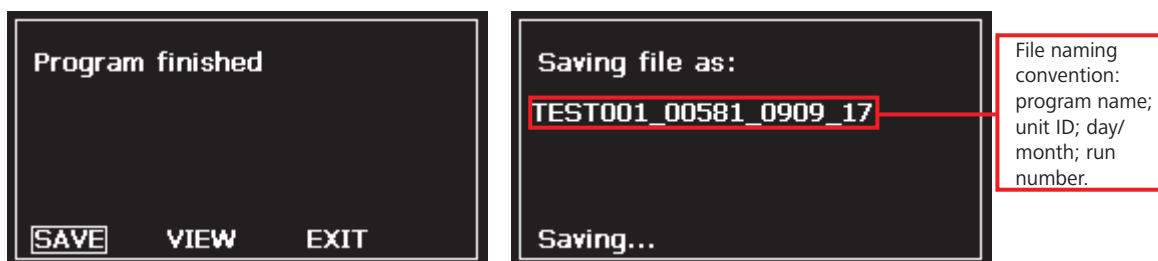
7. Data Analysis

7.1. Thermal Cycling

1. During a Thermal Cycling program, the fluorescence intensity of each well is measured after the first and last cycles. You may change from End-point detection to Real-Time detection in the “Options” menu by turning on “Real-Time mode” in the “System Settings” menu. You must agree to the disclaimer before Real-Time mode will be turned on.



2. At the end of a Thermal Cycling program, you may view the fluorescence results on the instrument's LCD screen. Fluorescence values are displayed on a scale from 001 to 999. Display value = (fluorescence last cycle - (minimum fluorescence))/2000 (rounded to the nearest whole number). If the result is <001, display "000".
3. When in Real-Time mode, data acquisition occurs following the Annealing/Extension step (Step 2) during 2-Temperature PCR, and following the Extension step (Step 3), during 3-Temperature PCR.
4. Selecting “Save” at the bottom of the screen saves the results to either the Flash card (2GB or less) or the USB memory key.



5. To graph the results, remove the Flash card or USB memory key from the instrument and insert it into the corresponding port of a PC.
6. Data is stored as a “.txt” file on the Flash card or USB memory key. The file will be saved with the following naming convention: AAAAAAA_UUUU_DDMM_RR.txt, where AAAAAA is the program name, UUUU is the unit ID number, DDMM are day and month, and RR is run of the day.



This data can ONLY be graphed using Spartan Graphing and Analysis software that is supplied with the instrument.

7. The User Manual for the Spartan Graphing and Analysis software is available for download at: <http://www.spartanbio.com/products/spartan-dx-12/manuals/>

7.2. Melt Curve

1. During a Melt Curve program, the fluorescence intensity of each well is measured after each temperature increment.
2. At the end of a Melt Curve program, select **“Save”** at the bottom of the screen to save the results to either a Flash card or a USB memory key on the instrument.
3. To graph the results, remove the Flash card or USB memory key from the instrument, and insert it into the corresponding port of a PC.
4. Melt Curve data is stored in the same file as the Thermal Cycling data. This data can also be graphed using Spartan Graphing and Analysis software.
5. The User Manual for the Spartan Graphing and Analysis software is available for download at: <http://www.spartanbio.com/products/spartan-dx-12/manuals/>