

# *Spartan* **DX-12**<sup>TM</sup>

## **Desktop DNA Analyzer**

Troubleshooting Guide

Version 3.10

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## 1. Troubleshooting Guide

This guide will provide you with the instructions and resources needed, should you encounter any problems with the Spartan DX-12™ instrument, the Spartan Graphing & Analysis software or your reagents.

### 1.1. Quick Reference Troubleshooting Table

Issue	Task
Instrument	Consult hardware troubleshooting section of this guide (see section 2)
	Run Status Report
	Contact your Reseller with Status Report information (see section 1.2)
	Contact Spartan Bioscience (support@spartianbio.com) with Status Report information
Graphing & Analysis Software	Consult software troubleshooting section of this guide (see section 3)
	Contact your Reseller
	Contact Spartan Bioscience (support@spartianbio.com)
Reagents	Consult PCR/Melt Curve troubleshooting section of this guide (see section 4)
	Contact your Reseller
	Contact Spartan Bioscience (support@spartianbio.com)

### 1.2. Instrument Self Test

If you feel that the instrument is not working to your expectations, navigate to the **“Options”** menu, and with a Flash Card or USB memory key inserted in to the unit, select **“Reports”**. The instrument will take 10-20 minutes to export a status report. Once the status report has been completed, the instrument will return to the welcome menu, with all required files (3 files, named UUUUUreportDDMMYY.CSV, .PNG, or .RGB where UUU represents unit number, and DDMMYY represent the date) saved to a folder named Status Report on the USB memory key. The Report can now be transferred to your PC and e-mailed to your reseller. If you have any questions or require help with this procedure, please contact your reseller.

No user-serviceable parts are inside. Do not remove covers that require tool access, or attempt to service the instrument yourself.

## 2. Troubleshooting Hardware

### Hardware

Issue	Task
Instrument does not turn on	<ul style="list-style-type: none"> <li>Ensure that the instrument is plugged in, and that the circuit in use has not been over-burdened.</li> </ul>
Instrument fails to save data	<ul style="list-style-type: none"> <li>Determine if the Flash Card or USB memory key was removed or changed while the program was running, data will not save correctly in this event.</li> <li>Try saving data on the USB memory key provided by Spartan Bioscience.</li> <li>To save your data, navigate to the <b>"Options"</b> menu, insert your USB memory key or Flash Card, and select <b>"Save Last Run"</b>. This will save an instrument data file, as well as a series of RGB files, which are the image files from which the data is generated. These are not intended for customer use, but may be requested by Spartan Bioscience for optical troubleshooting.</li> </ul>
Instrument fails to save data after it was left unattended and/or ran overnight	<ul style="list-style-type: none"> <li>Determine if the instrument went into Auto-off mode, if so, all your data should still be present in the instrument memory.</li> <li>Determine if there was a power failure during or following the run. <b>Note: In the event of a power failure to the unit, the data saved in the instrument memory, for the most recent run will be lost.</b></li> <li>To save your data (if there was no power failure), navigate to the <b>"Options"</b> menu, insert your USB memory key or Flash Card, and select <b>"Save Last Run"</b>. This will save an instrument data file, as well as a series of RGB files, which are the image files from which the data is generated.</li> </ul>
Spartan DX-12™ experiences a run abort error	<ul style="list-style-type: none"> <li>Ensure that if a custom process file was run it did not have values above 99°C, the instrument will abort if temperatures of above 105°C are attained.</li> <li>To retrieve your data, navigate to the <b>"Options"</b> menu, insert your USB memory key or Flash Card, and select <b>"Save Last Run"</b>.</li> </ul>
One or more wells consistently fail, or show significantly lower fluorescence levels	<ul style="list-style-type: none"> <li>Hard reboot the instrument (unplug and replug the instrument).</li> <li>Repeat the experiment and show consistency (i.e., demonstrate that it is not a chemistry problem).</li> </ul>

	<ul style="list-style-type: none"> <li>• Start a run and physically look into the wells to see if all of the LEDs are on and shining at the same intensity.</li> <li>• Run an instrument <b>“Status Report”</b> with empty tubes in the wells (see section 1.2), and send the results to your reseller.</li> </ul>
PCR fails, or shows increasing Cts in an inside out, or an outside in manner	<ul style="list-style-type: none"> <li>• Ensure that there are empty tubes present in all wells not running PCR reactions.</li> </ul>
The instrument takes a long time to reach temperature (greater than specified in the specification sheet)	<ul style="list-style-type: none"> <li>• Hard reboot the instrument (unplug and replug the instrument).</li> <li>• Make sure the room temperature is between 20 - 25°C.</li> <li>• Make sure the instrument has adequate ventilation area, and that nothing is blocking the air vents.</li> <li>• Check to see if the fan (at the rear of the instrument) is running.</li> </ul>
Instrument is unusually loud, or is making odd noises	<ul style="list-style-type: none"> <li>• Hard reboot the instrument (unplug and replug the instrument).</li> <li>• Make sure the instrument has adequate ventilation area, and that nothing is blocking the air vents.</li> <li>• Check to see if the fan (at the rear of the instrument) is clear of obstructions.</li> </ul> <p><b><i>Note: Ensure that the instrument is turned off and that there is no power running to it before attempting to remove any objects obstructing the fan.</i></b></p> <ul style="list-style-type: none"> <li>• Run an instrument <b>“Status Report”</b> with empty tubes in the wells, and send the results to your reseller (see section 1.2).</li> </ul>
Instrument user interface freezes	<ul style="list-style-type: none"> <li>• Hard reboot the instrument (unplug and replug the instrument).</li> </ul>

### 3. Troubleshooting Software

#### Software

Issue	Tasks
Cannot install Spartan Graphing & Analysis Software onto their lab PC	<ul style="list-style-type: none"> <li>• Determine if the minimum PC system requirements have been met.</li> <li>• Ensure that the user account being accessed has administrative privileges.</li> <li>• Contact your system administrator for help installing the program.</li> </ul>
Cannot locate/open data file	<ul style="list-style-type: none"> <li>• Ensure that the data is being loaded through <b>"Import"</b> and not <b>"Open"</b> functions of the software.</li> <li>• Verify file location.</li> <li>• Verify file extension (Import › .txt vs. Open › .sdx).</li> </ul>
Data is loaded as a straight line instead of as a Real-Time PCR plot	<ul style="list-style-type: none"> <li>• Your instrument is most likely in End-point mode, and you will be unable to get Real-Time data from this run. Ensure that you can agree to the Real-Time disclaimer and that the feature has been turned <b>"On"</b>.</li> </ul>
No melt curve data is shown	<ul style="list-style-type: none"> <li>• Ensure that the melt curve program was set to <b>"On"</b> in the instrument program file, and that all the disclaimers have been agreed to.</li> <li>• Verify that you are looking at the appropriate data tab.</li> </ul>
Unexpected positive amplification in second channel	<ul style="list-style-type: none"> <li>• Ensure that you are using manufacturer recommended fluorophores (see section 7.3.1 in the Spartan Analyzer Quick Reference Guide, or 12.3.1. ub the User Manual).</li> <li>• Refer to the Graphing &amp; Analysis Software User Manual section 5.2.4.</li> <li>• Infrequently, cross-talk may occur between fluorophores from one detection channel to another. In order to identify possible cross-talk issues, look at the relative fluorescence scales. If one is significantly greater than the other, what you are seeing is likely cross-talk and should be disregarded.</li> </ul>

## 4. Troubleshooting PCR/Melt Curve

### PCR/Melt Curve

Issue	Likely Causes	Potential Solutions
No amplification throughout entire reaction or very high Ct values	Hot-start enzyme not activated	Make sure to follow the manufacturer's instructions for activation of all hot start enzymes.
	Inefficient/lack of primer binding	Primer design is very important for successful PCR. There are many free internet resources to help you with this (for example Primer 3: <a href="http://frodo.wi.mit.edu/primer3/">http://frodo.wi.mit.edu/primer3/</a> ) Always design and test several sets of primers when designing an assay. Check for the appropriately sized PCR product band on an agarose gel, or by melt curve analysis (if T <sub>m</sub> is known).
	Amplicon too long	Optimal amplicon length for real-time PCR is 80-200 bp.
	Primers degraded	Thaw a fresh aliquot and repeat test. If available, determine primer integrity by running them on a denaturing polyacrylamide gel.
	Annealing step too short	Add time to the annealing step in 5 second increments up to 30 seconds (for amplicons up to 200 bp)
	Annealing temperature is set too high	Set your initial annealing temperature to be 5°C below calculated primer T <sub>m</sub> .
	Poor quality nucleic acid	Determine the quality of your template using spectrophotometry, look for a 260/280 ratio of between 1.6-2.0 for DNA or 1.8-2.0 for RNA. The presence of proteins or other contaminants within your nucleic acid sample may also inhibit your reaction. If necessary clean up or re-isolate your nucleic acids.
	Too much or too little nucleic acid template	Determine the quantity of your template using spectrophotometry. Starting amounts of template should be in the ng range for plasmid DNA and µg amounts for genomic DNA for initial reaction optimization.
Fluorophore/probe concentration/fluorescence is too low or too high	Ensure that your fluorophore is stored according to manufacturer's instructions and avoid exposing it to light, or freeze thaw cycles. High concentrations of DNA binding Dye (e.g., SYBR Green®) can be inhibiting, start with concentrations around 1.0X and adjust up or down by 0.1X until optimal concentration is achieved. Optimal probe concentrations can vary significantly. Follow manufacturer's suggested concentration.	

	Setup Error	Verify reagent concentrations & storage conditions. Take out fresh aliquots and repeat experiment. Use a checklist when setting up reaction master mixes.
	Inefficient probe binding	Verify primer & PCR efficiency using a DNA intercalating dye (such as SYBR Green®). Redesign probe (with a T <sub>m</sub> ~10°C above that of your primers) if primers work well.
	Primer concentration requires optimization	Primer concentrations can range from 0.1-1.0 µM. Vary primer concentrations by 0.2 µM up to a maximum total primer concentration of 1.0 µM.
	MgCl <sub>2</sub> concentration requires optimization	Increase MgCl <sub>2</sub> concentration by 0.25-0.5 mM increments to a maximum of 5 mM.
Non-specific amplification and/or primer dimers	Annealing temperature too low	Increase the annealing temperature in 1-2°C increments. Run a melt curve or Run results on an agarose gel or by to determine product specificity.
	MgCl <sub>2</sub> concentration requires optimization	Decrease MgCl <sub>2</sub> concentration by 0.25-0.5 mM increments to a minimum of 1 mM.
	Reaction setup at room temperature	Always setup reactions on ice before transferring tubes to pre-warmed instrument.
	Primers degraded	Thaw a fresh aliquot and repeat test. If available, determine primer integrity by running them on a denaturing polyacrylamide gel.
Fluorescence in the negative control	Reagent contamination	Take out new reagent aliquots and repeat. Never use master mix reagents and nucleic acid components in the same area.
	Setup contamination	Assemble all components of the master mix in a DNA free area, add in the nuceic acid components in a seperate area. Always thoroughly clean up setup areas with a product that degrades nucleic acids.
No amplification in positive control	Template degradation	Determine template integrity using PAGE. Take out a new template aliquot and repeat .

Data plots are very noisy/ messy	Using a fluorescent quencher	Using fluorescent quenchers such as TAMRA can increase sample background noise, reducing the signal to noise ratio. To avoid this use non-fluorescent quenchers, such as BHQ.
	High concentrations of template DNA	High concentrations of starting material can increase sample background noise, reducing the signal to noise ratio. Reduce starting concentrations of nucleic acid.
	Too much/too little DNA intercalating dye	Too much, or too little DNA intercalating dye can result in noisy plots. Vary your intercalating dye by $\pm 0.2X$ increments until the optimal concentration is achieved.  <b>Note: High concentrations of some intercalating dyes (such as SYBR Green®) may inhibit PCR.</b>

## 5. Return Procedures

- Should you have to return an instrument, please contact your reseller for instructions
- Before any unit can be returned, it must be decontaminated as described in section 18 of the User Manual.