



Real-time DNA Analyzer

Quick Reference Guide

Version 1.2

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Research Use Only

This instrument is for research use only and is not intended for clinical or diagnostic applications.

Disclaimer

PCR and real-time PCR processes are covered by patents issued and applicable in certain countries. Spartan does not encourage or support the unauthorized or unlicensed use of PCR or real-time PCR processes. Use of this instrument is recommended for persons that either have licenses to perform PCR and real-time PCR or are not required to obtain licenses.

Patents Pending

The Spartan DX™ is protected by patents pending in multiple geographic areas.

Trademarks

Spartan DX™ is a registered trademark of Spartan Bioscience Inc. All other trademarks are the sole property of their respective owners.



Please refer to the SpartanDX™ Version 1.1 Operator's Manual posted online at <http://www.spartanbio.com/manuals.asp>, for complete operating instructions and important safety information.

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Operating Instructions

Reaction Set-up

A. Two-temperature thermal cycling

The Spartan DX™ contains two fixed-temperature heat blocks and is designed to run two-temperature PCR programs. A conventional three-temperature PCR protocol has three steps: denaturation, annealing, and extension. A two-temperature protocol combines the annealing and extension steps into a single step.

Each gene target is unique and may require optimization of the cycling program. To determine the appropriate two-temperature program for your application, follow these steps:

1. Design primers with matching melting temperatures (T_m) of 60-68°C, and amplicon size of < 300 base pairs (bp). For fastest cycling speeds, the primers should have T_m 's of 65-68°C, and amplicon size < 150 bp. If primers and amplicons do not meet these criteria, then amplification may be unsuccessful.
2. Set the annealing/extension temperature at the calculated T_m .
3. Set the annealing/extension time to 45 seconds.
4. Start with the following denaturation temperatures and times:

DNA Source	Denaturation Temp	Initial Time	Cycling Time
Human/Mammalian	95°C	2.5 min	30 s
Bacterial/Viral	95°C	1 min	30 s

5. To optimize the speed of your reaction, consult the "Program Selection Chart" in Appendix A to determine the fastest dwell times to achieve different combinations of denaturation and annealing/extension temperatures.

B. Detection chemistries

The Spartan DX™ is a semi-quantitative instrument, accurate within ± 1 cycle.

1. For SYBR Green I (Invitrogen, Cat. No. S7563), we recommend a final concentration of 0.2-0.7X. The optimal concentration is 0.4X. For further details, see Application Note 009 (www.spartanbio.com/application_notes.asp).
2. TaqMan® probes are compatible with the Spartan DX™. We recommend using probes with BHQ-1 (Black Hole Quencher®) non-fluorescent quenchers. For further details, see Application Notes 005 and 012.

C. Reaction tubes

The Spartan DX™ is designed to work with standard 0.2 ml thin-wall flat cap PCR tubes. PCR tubes with thinner side walls have faster heating and cooling kinetics. We recommend thin-wall tubes from VWR (Cat. No. 53550-106). For further details, see Application Note 017.

D. Reaction volumes

Reaction volumes of 10-50 μ l may be used. For best results, we recommend a reaction volume of 20 μ l. For further details, see Application Note 008.

E. Reaction setup

1. Prepare real-time PCR mixture.
2. Aliquot mixture into thin-wall flat cap PCR tubes.
3. Overlay reaction mixture with mineral oil (e.g. Biotools, Cat. No. 20.032).



For faster cycling speeds, we recommend 20 μ l reaction volumes with a 15 μ l overlay of mineral oil.

Run Program

A. Warm up

1. Turn the instrument on by pressing center button on the keypad.
2. Press center button again and select "RUN/EDIT" to start the unit heating to default temperature.
3. It will take 10 minutes to warm up and equilibrate to the default block temperatures of 95°C and 37°C from room temperature.
4. Once a program is selected, the instrument will heat to the selected program's block temperatures.

B. Program selection

1. Use outside keypad buttons to move between program numbers. Press center keypad button to select a program.

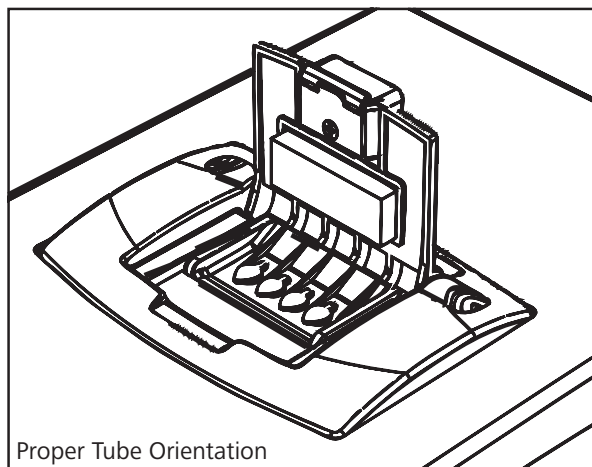
C. Program settings

The Spartan DX™ is set up to run an initial denaturation step followed by a 2-temperature thermal cycling program. Default denaturation temperature is 95.5°C. Annealing and extension steps are combined together and performed at a default temperature of 55.5°C.

1. To modify a setting, use outside keypad buttons to move between temperatures, dwell times, and cycle number.
2. Press center keypad button to select the setting to be modified.
3. Use outside buttons to increase or decrease the setting values.
4. Press center button again to save the modified setting.
5. Save a modified program by selecting "SAVE" using center button.

D. Run program

1. Insert reaction tubes into the device so that the tube hinge points to the rear of the instrument. Close the instrument lid.
2. Run the program by selecting "START".
3. You may select "PAUSE" or "STOP" at any time during the run. **NOTE: You may need to redo the reaction if you pause or stop for more than 5 seconds.**
4. When run is finished, select "YES" to save results to the device's Flash Card.
5. Results are saved in standard ".csv" format with a date-and-time stamp. To update the date and time, select "OPTIONS" and adjust the date and time values.
6. After 30 min of inactivity, the display screen will turn off. After 4.5 hours of inactivity, the instrument will turn off. Note that unsaved data will remain in memory until specifically canceled by the user.



Run Program (continued)

E. Reverse Transcription

For RNA analysis, the instrument is capable of performing an initial reverse transcription (RT) step, followed by a thermal cycling program. To set up an RT program, follow these steps:

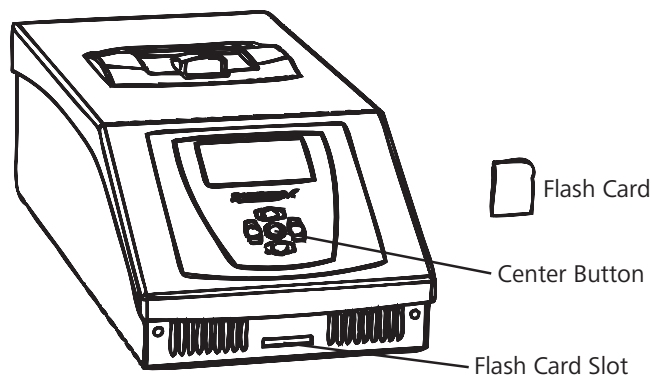
1. From the main menu, select "RUN/EDIT".
2. Select the "RT" program from the program menu.
3. In the "RT" program, set the temperature and dwell times for reverse transcription and heat inactivation of the RT enzyme.
4. Select "START" to accept the RT program. This will select the RT program, and send you back to the program menu.
5. In the program menu, the "RT*" symbol indicates that the RT program has been selected.
6. Select the thermal cycling program to be performed after the RT program. You may select from Programs #1-8.
7. In the thermal cycling program, select "START" to begin the run. The RT program will be performed first, followed by the thermal cycling program.

F. Data Analysis

The fluorescence intensity of each well is measured after every cycle and displayed on the screen. If you only desire fluorescence measurements for the first and last cycles (end-point detection), then select the "OPTIONS" menu and toggle the "REAL-TIME" setting to "OFF".

To plot a graph of fluorescence intensity versus cycle number:

1. At the end of the run, select "YES" when asked to "SAVE RESULTS?" to save data onto the Flash Card.
2. Eject Flash Card from front of device.
3. Insert Flash Card into appropriate slot in your computer.
4. Data is stored as a ".csv" file on the Flash Card. This data may be graphed using a Microsoft Excel® macro program which is provided with the Spartan DX™. This software is downloadable from <http://www.spartanbio.com/support.asp>.
5. In Excel®, set your macro security settings to "Medium" by going to the "Tools" menu, selecting "Macro", and then selecting "Security". Set the "Security Level" to "Medium" and click "OK".
6. Double click on the Spartan GraphApp macro software. This will launch Excel® and prompt you to "Enable Macros" to run the macro.
7. In the Spartan GraphApp software, select "Load Flash Card Data" to browse to the .csv file containing your data. The software will automatically load your data and graph your results.
8. To edit the sample names, select the "Summary" tab (Sample 1 = Well 1, numbered from left to right).
9. Save your graphed results using the "Save" or "Save As" commands in the "File" menu. Note that saving the file will not alter the Spartan GraphApp program.



Appendix A: Program Selection Chart

Instructions

1. Calculate the melting temperature (T_m) of your PCR primers using online software from IDT (<http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/>).
2. If you know the melting curve profile of your PCR product, then choose a target denaturation temperature that is to the right side of this curve. This temperature is usually lower than 92°C. If you do not know the melting curve profile, then use 92°C as the target value for your desired denaturation temperature.
3. Use the "Program Selection Chart" below to determine the optimal temperature settings and dwell times for the Spartan DX™ based on your calculated T_m and desired target denaturation temperature.
4. For the initial denaturation temperature and dwell time, a setting of 95°C for 2.5 min is usually sufficient for human or mammalian genomic DNA. For bacterial or viral DNA, a setting of 95°C for 1 min is usually sufficient. Note that hot-start enzymes may require a longer initial denaturation time.

Example

- If your calculated primer T_m is 61°C, then use 61°C as your desired annealing/extension liquid temperature.
- If your desired denaturation liquid temperature is 92°C, then the chart below recommends that you set the Hot Block at **95°C** with a dwell time of **30 s** and the Cold Block at **53°C** with a dwell time of **26 s**.
- These settings will result in the liquid temperature cycling between 92°C and 61°C.

		Hot Block setting of 95°C ¹			
Cold Block setting ¹	Liquid Temp ²	86-87°C	88-89°C	90-91°C	92-93°C
50°C	53-54°C	20/38 s	23/29 s	27/40 s	34/42 s
	55-56°C	19/36 s	22/37 s	26/38 s	33/40 s
	57-58°C	18/20 s	21/29 s	25/31 s	31/32 s
53°C	59-60°C	17/27 s	20/28 s	24/30 s	30/31 s
	61-62°C	18/22 s	21/23 s	25/25 s	30/26 s
57°C	63-64°C	16/23 s	19/24 s	23/26 s	29/27 s
	65-66°C	15/19 s	18/20 s	22/22 s	28/23 s
	67-68°C	13/15 s	16/16 s	20/18 s	26/19 s

¹Times are shown as: Dwell time on Hot Block / Dwell time on Cold Block.

²Liquid temperatures assume a reaction volume of 20 µl, a mineral oil overlay of 15 µl, and the use of thin-wall 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106).