

Spartan **DX-12**TM

Desktop DNA Analyzer

Graphing & Analysis Software
Manual
Version 3.10

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Definitions / Abbreviations

RT – Reverse Transcription

SDX – Spartan DX file

STD – Spartan standard curve

ID – Identification

TXT – Text file

SD Card – Secure Digital Card

USB – Universal Serial Bus

+/- df/dT – positive (+) or negative (-) first derivative

CP – Crossing point

CT – Threshold cycle

PCR – Polymerase Chain Reaction

PC – Personal computer

ΔFI – Delta fluorescence

IPC – Internal Positive Control

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 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, and 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>• <i>Italic</i> text indicates new or important words and is also used for emphasis. For example: <i>This data can <u>ONLY</u> be graphed using Spartan Graphing & Analysis software that is supplied with the instrument.</i>• Bold text &/or <u>underlined</u> text is also used for emphasis. See above example.

2. Preface

2.1. System Requirements

2.1.1. Hardware

800 MHz x 86 processor or higher

1024 x 768 display or higher

512 Meg of RAM minimum

2.1.2. 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 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 software”**. Upgraded versions of software can be installed without the need to uninstall previous versions.

2.2. Support

For more information or help with Spartan Graphing & Analysis software, contact Spartan Bioscience by e-mail at support@spartanbio.com.

3. About Spartan Graphing & Analysis Software

Spartan Graphing & Analysis software is a full-featured analysis package for data produced by the Spartan DX-12™ instrument. 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. Spartan Graphing & Analysis software automatically analyzes data from imported data files, displays results in a convenient graphical form, and provides the option to adjust analysis settings to your preferences. The software is organized into three tabs: Run Data tab, Quantification tab, and Melt Curve tab. Each tab has a different function, and each of these functions is described in the following sections.

Note - The default display in Spartan Graphing & Analysis software is the Run Data tab. 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).

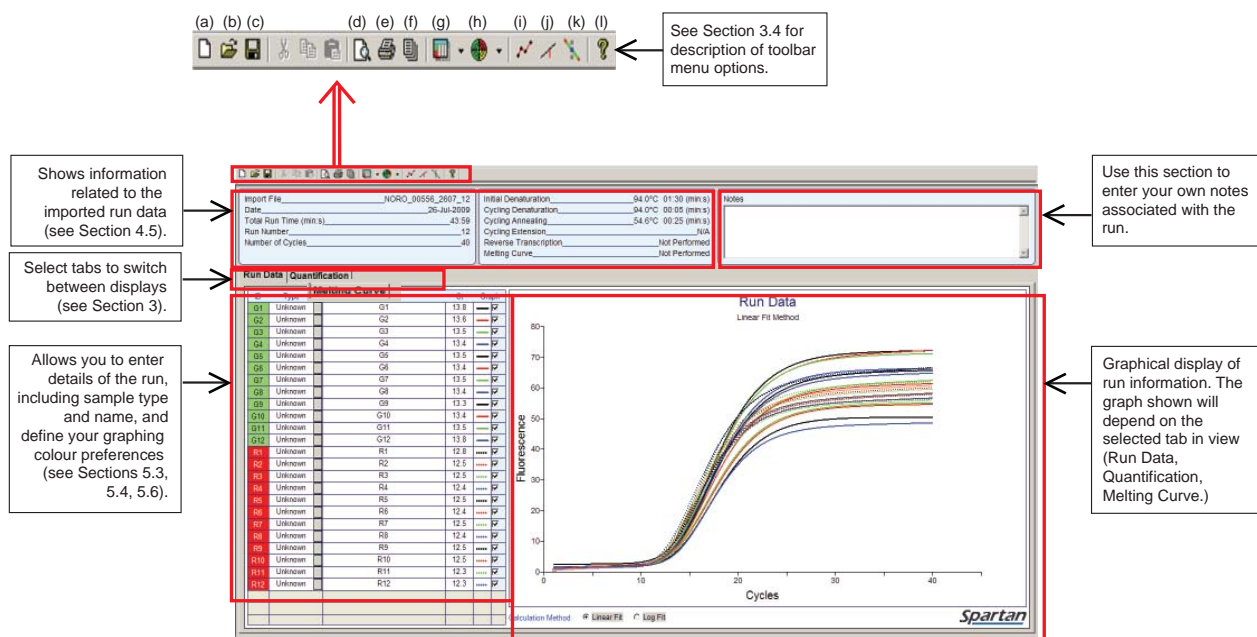


Figure 1. Default window display (Run Data tab) in Spartan Graphing & Analysis software.

3.1. Run Data Tab (see Section 5 - Using the Run Data Tab)

The Run Data tab is the main window and is displayed when the program is opened. This tab displays Real-Time or End-Point PCR data graphically as Fluorescence versus Cycles.

In this tab you may:

- *View experimental data as Fluorescence vs. Cycles.*
- *Choose the calculation method used for data analysis (Log fit or Linear fit).*
- *Automatically or manually select fluorescence thresholds for real-time data.*
- *Define Sample Type and Sample Name (changes made will be applied to other tabs).*

3.2. Quantification Tab (see Section 6 - Using the Quantification Tab)

The Quantification tab is available when 3 or more cycles of PCR data points are present in the imported data file. This tab is used to plot standard data and interpolate concentrations

of unknown samples.

In this tab you may:

- *Define concentrations of standards.*
- *Plot standard curves from internal standards (i.e., included in the current run).*
- *Save standard curves and import previously generated external standard curves to compare with current run data.*

3.3. Melting Curve Tab (see Section 7 - Using the Melting Curve Tab)

The Melting Curve tab is displayed when melt curve data is available in the imported file. This tab is used to plot melting peak or melting curve profiles.

In this tab you may:

- *View melting profiles as Melt Peaks or Melt Curves.*
- *View melt peaks as $-dF/dT$ or $+dF/dT$.*
- *Automatically or manually define one or multiple melting peaks.*

3.4. Overview of the Toolbar Menu:

The following toolbar menu options are available in Spartan Graphing & Analysis software (Figure 1):

- a) Import Raw Data – load data from a Spartan DX-12™ run in the form of an encrypted .txt file (Section 4.1).
- b) Open Data File – load previously saved data in the form of a .sdx file (Section 4.3).
- c) Save – save data in the form of a .sdx file (Section 4.2).
- d) Print Preview – view the current tab in print layout.
- e) Print – print the current tab (Run Data, Quantification, Melting Curve) in view (Section 4.9).
- f) Print Reports – print multiple tabs (Section 4.10).
- g) Edit Well Information Templates – open the Edit Well Information Templates dialogue box (Section 8.1).
- h) Edit Calling Results Definitions – open the Edit Calling Results Definitions dialogue box (Section 9.4.1).
- i) View data points – overlay raw data points on the Run Data graphs (Section 5.8).
- j) View graph thresholds – display threshold cycle (Ct) markers as vertical lines on the Run Data graph (Section 5.9).
- k) View replicates in graph – display replicate samples (i.e., standard samples with the same concentration) on the Quantification graph (Section 6.6).
- l) About – display information about Spartan Graphing & Analysis software.

4. Basic Operations

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


- A) **“Import Raw Data”** (see Section 4.1 – 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 an .sdx file.
- B) **“Open Data File”** (see Section 4.3 – Opening an .sdx File) - this option is used to open a previously saved Spartan Graphing & Analysis file format 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

UUUU is the unit ID number, DDMM are the day and month, and RR is the run number 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>.

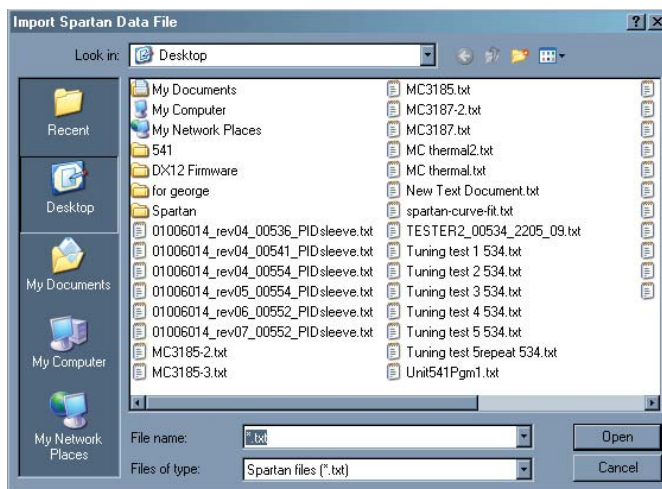



Figure 2. Import Data File dialogue window

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).

4.2. Saving Data

Data imported into the software from a data file can be saved as a Spartan Analyzer file format (.sdx extension), that 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 a Data File”** 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 (Figure 3).

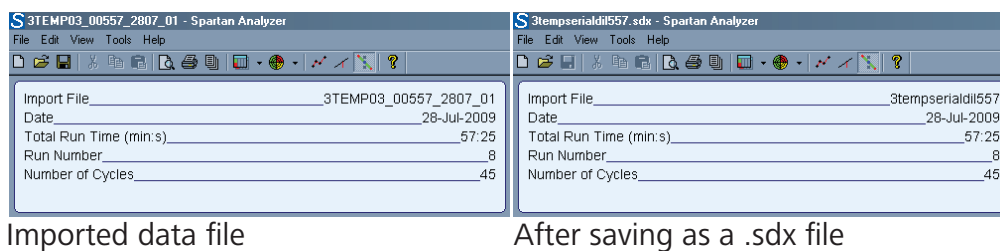



Figure 3. Program title bar

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

4.4 Viewing File ID and Run Information

Details of the imported data file, including run information, are displayed in the header of the Spartan Graphing & Analysis software window, below the toolbar. These include:

- Import File – Name of the imported data file.
- Date – Date of the run.
- Total Run Time (min:s) – Time taken to complete the run.
- Run Number – Run number of the day.
- Number of Cycles – Number of cycles performed during the thermal cycling stage of the run.
- Run Conditions – Reverse Transcription, Denaturation, Annealing, Extension and Melt Curve parameters.

Note: *If a multi-step melt curve is performed, the displayed step size will be the*

smaller of the two increment sizes used.

The Notes section of the header can be used to enter your own notes associated with the run.

4.5. Exporting Graphs

In all tabs of the software, the graph can be copied to the clipboard or exported as a JPEG.

- To copy or export a graph, right mouse-click on the graph area and select **“Copy Window to Clipboard”** or **“Export Window as JPEG”** from the pop-up menu.

4.6. Exporting Program Window Captures

In all tabs of the software, the program window can be copied to the clipboard or exported as a JPEG.

- To copy or export the program window, select **“Tools”**, then **“Copy Window to Clipboard”** or **“Export Window as JPEG”** from the menu bar.

4.7. Exporting Raw Data

Raw data can be exported from the Spartan Graphing & Analysis software into a .txt file. In all tabs of the software, the program window can be copied to the clipboard or exported as a JPEG.

- To Export raw data, select **“File”**, and then **“Export Raw Data”** from the menu bar.

The exported data will be saved with the naming convention: YourFileName.sdx.txt.

4.8. Copying and Pasting Data


In all tabs of the software, the well information section can be copied to the clipboard and pasted into data processing or spreadsheet software.

- To copy data, select **“Edit”**, then **“Copy”** from the menu bar.

The copied data can then be pasted directly into the recipient program.


4.9. Printing Reports

To print a copy of the current tab in view (Run Data, Quantification or Melting Curve), use one of the following options:

- A) Select **“File”** then **“Print”** from the toolbar menu; or
- B) Click on the Print icon  on the toolbar; or
- C) Use the Hotkey <Ctrl + P>.

4.10. Printing Multiple Reports

To print Run Data, Melting Curve and Quantification Analyses as a multiple report, use one of the following options:

- A) Select **“File”** then **“Print Reports”** from the toolbar menu; or
- B) Click on the Print Reports icon  on the toolbar.

A dialogue box will be shown (Figure 4), allowing you to select which information is to be included in the report.

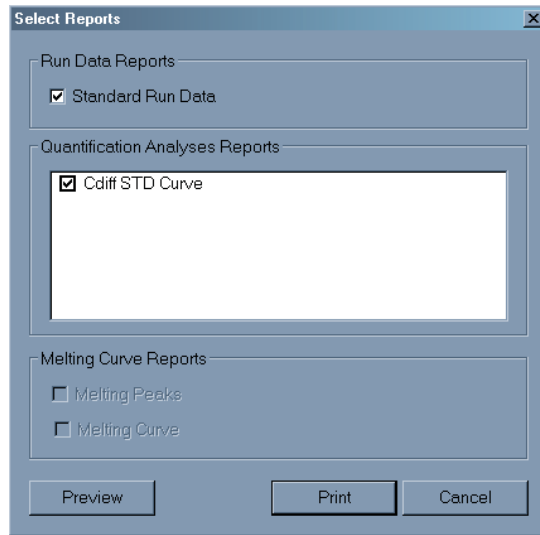


Figure 4. Print Reports dialogue box

5. Using the Run Data Tab

The Run Data tab displays either Real-Time or End-Point PCR data, as specified by the imported data file. The Run Data tab will always be displayed as the default screen. If there is no thermal cycling data in the imported file, the following message will be displayed in this tab: “Thermal Cycling data not available”.

5.1. Run Data Tab Sections

This tab contains two main sections (Figure 5): Well information section and Graph section.

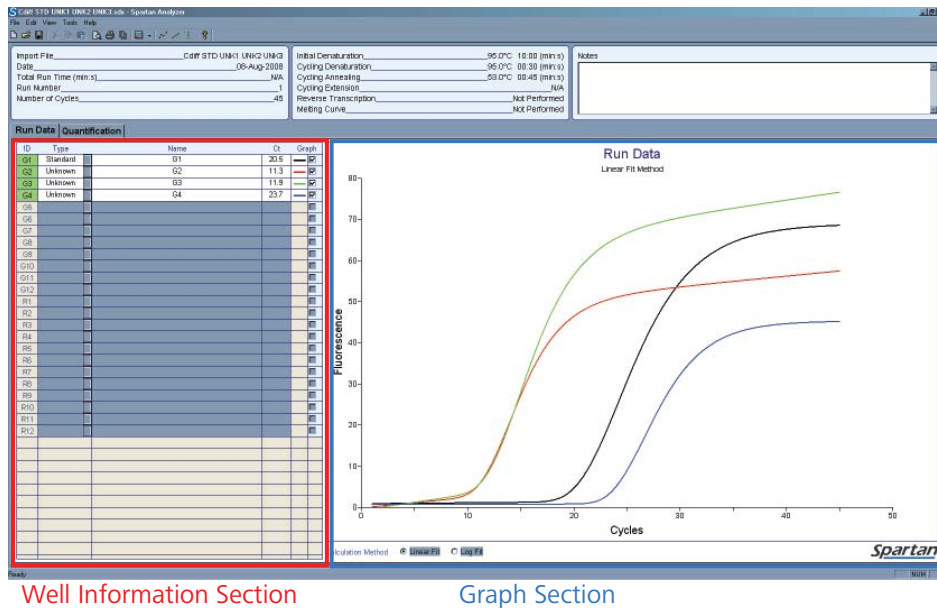


Figure 5. Run Data tab

5.1.1. Well Information Section

The Well Information Section allows you to input sample details, to specify whether or not to display the data for each well on the adjacent graph, and to select the colour of the plotted lines. Each row in the table corresponds to a specific channel and well on the Spartan DX-12™. The ID column displays the channel (G – Green, R – Red) and well number (1 – 12).

5.1.2. Graph Section

The Graph Section of the Run Data tab displays thermal cycling data for the wells you have selected (by checking the boxes) in the well information section. Run data for green channel wells are plotted using solid lines and run data for red channel wells are plotted using dotted lines.

The graph will display either Real-Time or End-Point data (Figure 6), as specified by the data in the imported file. Real-Time data is displayed as fluorescence at each cycle and End-Point data is displayed as fluorescence at the first and last cycles.

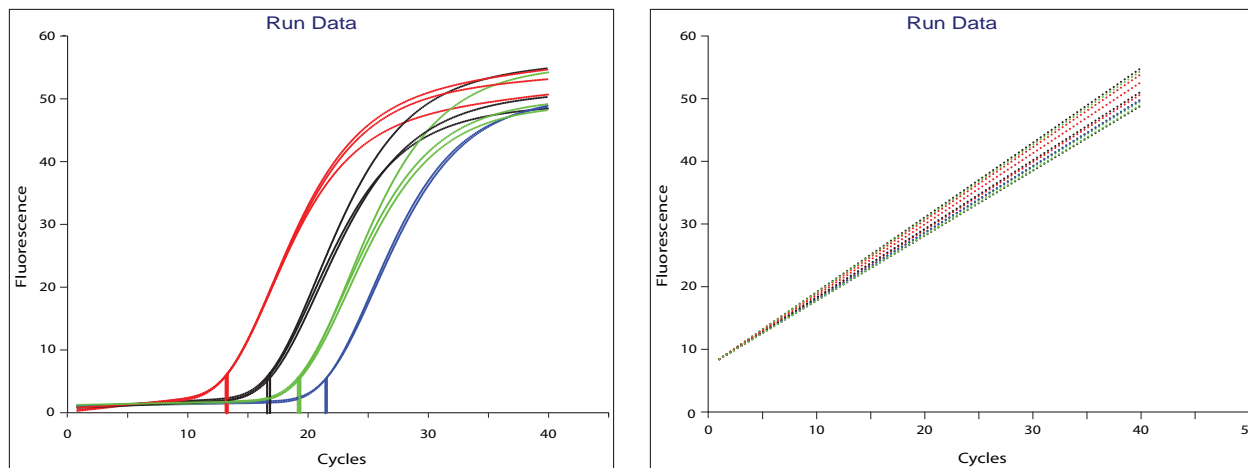


Figure 6. Real-Time and End-Point data displays in the Run Data tab

5.2. Understanding Run Data Analysis

5.2.1. Data Handling During Import

For each green and red channel well, raw fluorescence data are baseline subtracted and normalized by the software, using the following equation:

$$Fl = \frac{(Fl_{raw} - Fl_{min})}{1000} + 1$$

Where:

Fl = Normalized fluorescence value at each cycle (displayed on the Run Data graph)

Fl_{raw} = Raw fluorescence value at each cycle

Fl_{min} = Minimum fluorescence value across all cycles

5.2.2. Curve Fitting

Curve fitting of Real-Time data is performed using a 6 parameter sigmoidal fit process, based on the following equation:

$$y = y_0 + lx + A \left(1 + \exp \left(- \frac{(x - x_0)}{b} \right) \right)^{-f}$$

Where:

y_0 = y offset

l = tilt of curve

A = amplitude

x_0 = position of ramp

b = parameter determining slope of the ramp

f = asymmetry of curve

Artifacts (e.g., spikes, outliers) in the data are automatically identified by the software and removed. Any data point that satisfies the following condition is considered as an outlier:

$$|y_n - f(x_n)| > 5 * \text{Average}(|y_n - f(x_n)|)$$

Where:

n = cycle number

y_n = actual fluorescence value

$f(x_n)$ = expected fluorescence value based on the sigmoidal curve fit

5.2.3. Ct Calling

The Threshold Cycle (Ct) corresponds to the cycle at which there is the first detectable increase in fluorescence above baseline. Spartan Graphing & Analysis software calculates these values automatically for each red and green channel well. In brief, the software identifies the Ct as the point on the Real-Time data curve where acceleration of the fluorescence signal reaches maximum. This will always be in the exponential phase of the Real-Time data curve.

For each green and red channel well, Ct values will not be calculated by the software if:

- Data does not reach a minimum fluorescence level.
- The software determines the fluorescence signal to be cross-talk from the opposing channel.

5.2.4. Color Compensation of Data

The Spartan DX-12™ instrument can simultaneously detect signals from two optical channels. Cross-talk between fluorophores may occur from one detection channel to another. To prevent misleading results, the software will not call Ct values for a particular channel and well if the observed increase in fluorescence is determined to be due to cross-talk from the opposing channel.

5.3. Editing Sample Types

To specify the sample type for each well and channel, use one of the following options:

- Click once on the sample type (displayed in the Type column of the well information section), then use the hot keys displayed in the table below to set the sample type.
- Click on the drop-down selection button at the right side of the Type column.

The following sample types may be specified:

Type	Keys	Description
Empty	E or 1	The imported data file contains data for the well but you have indicated that the well is empty or the well contains no data. When selected, all other fields in this row will be disabled.
Standard	S or 2	The well contains data for a standard. Standard types allow for subsequent input of concentration/copy number in the Quantification tab of the software.
Unknown	U or 3	The well contains an unknown / test sample.
Positive	P or 4	The well contains a positive control sample.
Negative	N or 5	The well contains a negative control sample.

Note that sample types specified in the Run Data tab will be applied to the Quantification and Melt Curve tabs.

5.4. Editing Sample Names

The sample names associated with each well default to the well ID (G1, G2, etc. or R1, R2, etc.). To edit these:

- Click once on the sample name (displayed in the Name column of the well information section) and type the new name. You may enter a name up to 60 characters.

When the name field is not wide enough to display the entire name, the complete name can be displayed as a tool tip when the mouse is floated over the field.

The sample names you enter into the well information section of the Run Data tab will also be applied to the Quantification and Melting Curve tabs in Spartan Graphing & Analysis software.

Note that sample names specified in the Run Data tab will be applied to the Quantification and Melt Curve tabs.

5.5. Choosing the Calculation Method for Run Data Analysis

The threshold values displayed for each well in the well information section are calculated automatically by the Spartan graphing & Analysis software. The values displayed are associated with the calculation method used in performing analyses.

5.5.1. Real-Time Data

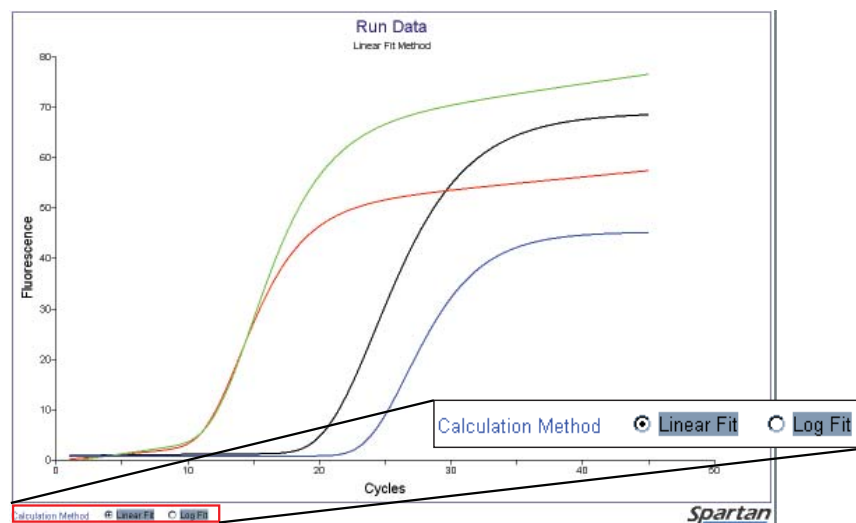


Figure 7. Calculation method option boxes in the Run Data tab

If the Run Data tab displays Real-Time data, the calculation method can be selected as “**Linear Fit**” or “**Log Fit**” by checking the option boxes displayed in the Graph section (Figure 7). The Linear Fit method is used as the default for newly imported data.

When the Linear Fit method is selected, the Threshold Values column will display Ct (Threshold Cycle) values. These values are calculated automatically by the Spartan Graphing & Analysis software as the cycle at which acceleration of the fluorescence signal reaches maximum (see

Section 5.2.3 – Ct Calling).

When the Log Fit method is selected, the column will display CP (Crossing Point) values. In Log Fit mode, it is possible to manually enter CP thresholds (see Section 5.10 – Manually Entering Crossing Point Thresholds).

5.5.2. End-Point Data

If the Run Data tab displays End-Point data, no calculation method options will be available, and the column will display ΔF_I (change in fluorescence). This is defined as the difference in fluorescence between the first and last cycles of PCR.

5.6. Changing Data Displayed on the Run Data Graph

To change the data displayed on the graph:

- Select or unselect the check boxes for each well (in the Graph column of the well information section).

To quickly change which graphs are displayed, the following functions may be used:

- CTRL + mouse click on a single box will uncheck all other boxes.
- SHIFT + mouse click on any box will select all boxes.

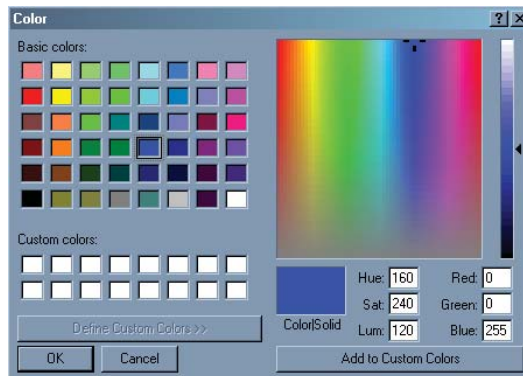


Figure 8. Edit colours dialogue box

To change the graphing colour for each well:

- Click once on the coloured line in the “Graph” column of the well information section. A dialogue box will appear (Figure 8), allowing you to define your colour preferences.

5.7. Editing the Run Data Graph Title

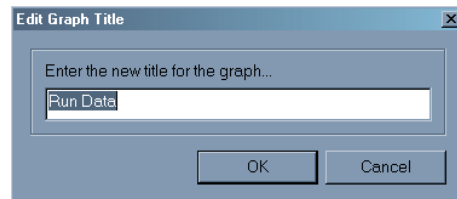



Figure 9. Edit graph title dialogue box

The main graph title defaults to “Run Data”. The subheading displays the calculation method you have chosen for analysis (Linear Fit or Log Fit). To edit the main graph title:

- Double-click with the mouse on the text and enter the new name into the dialogue box (Figure 9).


5.8. Displaying Raw Data on the Run Data Graph

The raw data (i.e., fluorescence value at each cycle) can be overlaid on the Run Data graph. To display raw data points, use one of the following options:

- A) Select “**View**” then “**Graph Data Points**” from the toolbar menu; or
- B) Click on the View Data Points icon “” on the toolbar.

5.9. Displaying Threshold Markers on the Run Data Graph

If you have chosen “**Linear Fit**” as the calculation method for analysis of Real-Time PCR data (see section 5.5 – Choosing the Calculation Method for Data Analysis), you may opt to view the threshold cycle (Ct) markers as vertical lines on the graph. To display threshold markers, use one of the following options:

- A) Select “**View**” then “**Graph Threshold Markers**” from the menu; or
- B) Click on the View Graph Thresholds icon “” on the toolbar.

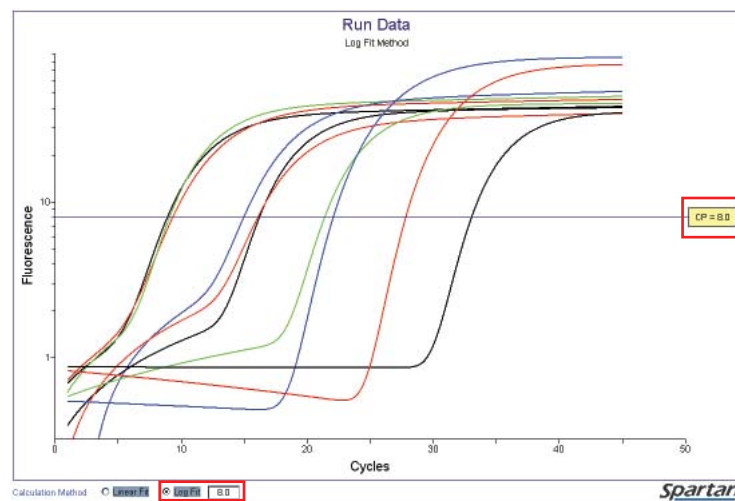


Figure 10. Manually entering CP Thresholds

5.10. Manually Entering Crossing Point Thresholds

If you have chosen “**Log Fit**” as the calculation method for analysis of Real-Time PCR data (see section 5.5 – Choosing the Calculation Method for Data Analysis), a horizontal line and label box (CP) will be displayed on the graph (Figure 10). This will allow you to manually set a crossing point (CP) threshold.

There are two ways to manually enter CP thresholds:

- A) Drag the line to the desired fluorescence value; or

B) Enter a value into the field adjacent to the Log Fit check box displayed below the graph.

CP thresholds should be chosen within the exponentially growing log-linear portion of Real-Time data curves (when sufficient product has been detected above background).

5.11. Changing the Run Data Graph Axes Scales

5.11.1. Fluorescence Axis

The default minimum limit, maximum limit and tick intervals of the fluorescence axis are automatically determined by the extreme values of the data displayed. These settings can be manually adjusted using one of the following options:

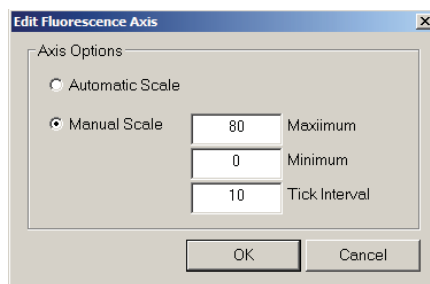


Figure 11. Edit Fluorescence axis scale dialogue box

- A) Right mouse-click on the axis and select **“Edit Scale”** from the menu; or
- B) Double click on the axis, then select **“Manual Scale”**

Enter values into the pop-up dialogue box (Figure 11). Selecting **“Automatic Scale”** will adjust settings to the default values.

The fluorescence axis scale can also be adjusted quickly by clicking once and scrolling with the mouse wheel. This may be helpful for accurate manual setting of the CP threshold value when using the Linear Fit calculation method (see section 5.10 – Manually Entering Crossing Point Thresholds).

5.11.2. Cycle Axis

The Cycle axis scale is automatically determined by the data available in the imported data file and cannot be edited.

6. Using the Quantification Tab

The Quantification tab is displayed when 3 or more cycles of PCR data points are present in the imported data file.

6.1. Quantification Tab Sections

This tab contains two main sections (Figure 12) – the Well Information section and the Graph section.

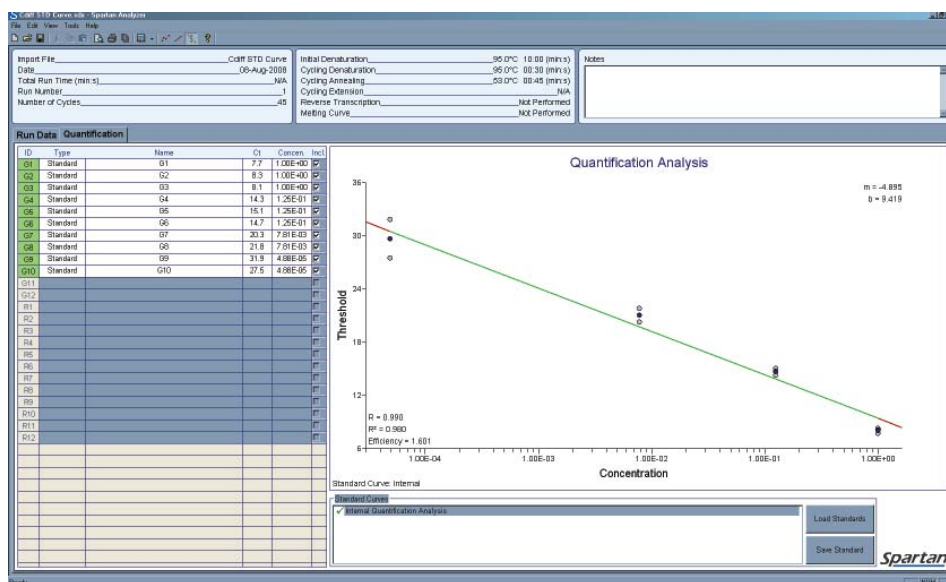


Figure 12. Quantification tab

6.1.1. Well Information Section

The well information section allows you to input concentrations of Standard samples and to specify which Standard samples are used for quantification analyses. Each row in the table corresponds to a specific channel and well on the Spartan DX-12™. The ID column displays the channel (G – Green, R – Red) and well number (1 – 12).

Sample Name and Sample Type can not be edited in the well information section of the Quantification tab, these are linked to the Run Data tab information.

6.1.2. Graph Section

The Graph section of the Quantification tab displays data for the currently selected standard curve (internal or external) and the wells you have selected (by checking the boxes) in the well information section.

6.2. Overview of Standard Curves

Standard curves plot concentration of Standard samples against threshold values (CP or Ct) and are used to quantify Unknown samples. Internal or external standard curves may be used for data analysis.

- An internal standard curve is generated from Standard samples included in the current run.
- An external standard curve is imported from a previously saved internal standard

curve and requires the use of a Calibrator sample.

Available standard curves are displayed in the Standard Curves selection box at the bottom of the Quantification Graph section (Figure 13).



Figure 13. Standard Curves selection box

Standard curves are plotted as the best linear fit to two or more unique data points. The graph axes are automatically determined by the maximum and minimum limits of the data and cannot be edited. The Concentration axis is displayed as a Log10 scale and the Threshold scale is displayed as a linear scale (Figure 14).

Points on the standard curve are colour coded as follows:

- Blue filled circles - points used to create an internal standard curve.
- Grey filled circles - replicates used to generate the standard curve.
- Red filled circles - points used to calibrate an external standard curve.
- Open squares - calculated points (i.e., Unknown samples).

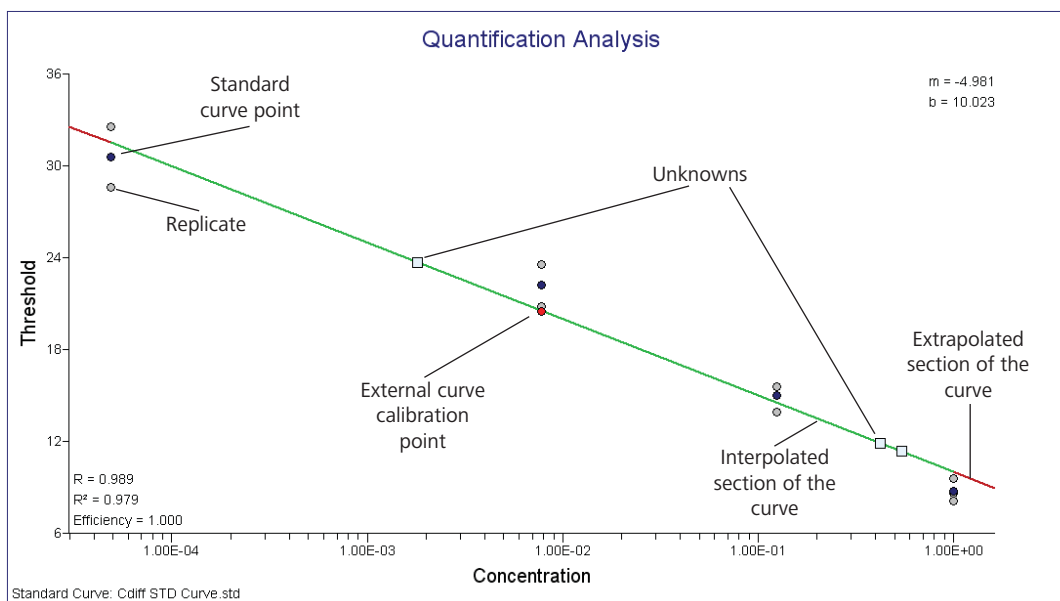


Figure 14. Standard Curve

6.3. Understanding Quantification Data Analysis

6.3.1. Standard Curve Fitting

Standard curves are plotted as the best linear fit to two or more unique data points. Each point consists of coordinate values for the cycle threshold and the Log10 of the concentration. When only two points are used, the line calculation is straight forward. When more than two points are available the line is calculated using the following equations:

Measure	Equation
Regression Line	$Y = mX + b$
Slope	$m = \frac{SP}{SS_x}$
Sum of Products (SP)	$SP = \sum XY - \frac{\sum X \sum Y}{N}$
Sum of Squares (SS)	$SS_x = \sum X^2 - \frac{(\sum X)^2}{N}$
Intercept	$b = \bar{Y} - m\bar{X}$
Mean Y	$\bar{Y} = \frac{\sum Y}{N}$
Mean X	$\bar{X} = \frac{\sum X}{N}$

The slope (m) and intercept (b) of the standard curve are displayed in the Graph section of the Quantification tab.

6.3.2. Correlation

The Pearson correlation coefficient (R) is calculated using the following equations:

Measure	Equation
Pearson Correlation (R)	$R = \frac{SP}{\sqrt{SS_x * SS_y}}$
Sum of Products (SP)	$SP = \sum XY - \frac{\sum X \sum Y}{N}$
Sum of Squares X	$SS_x = \sum X^2 - \frac{(\sum X)^2}{N}$
Sum of Squares Y	$SS_y = \sum Y^2 - \frac{(\sum Y)^2}{N}$

The correlation coefficient (R) and coefficient of determination (R²) are displayed in the lower left corner of the graph section.

6.3.3. Efficiency

Efficiency of the reaction is calculated from the fitted standard curve, using the following equation:

$$Efficiency = 10^{-1/Slope}$$

(e.g., If slope = -3.3 → Efficiency = 2).

6.3.4. Calculating Concentrations of Unknown Samples

By determining where an Unknown sample's threshold value falls on the standard curve, the software can determine the initial concentration of target DNA in the sample. Concentrations of Unknown samples are calculated from the fitted standard curve, using the following equations:

Measure	Equation
$\text{Log}_{10}(\text{concentration})$	$\text{Log}_{10}(\text{concentration}) = X = \frac{Y - b}{m}$
Concentration	$\text{concentration} = 10^x$

6.4. Generating Standard Curves

Standard curves plot concentration of Standard samples against threshold values (CP or Ct) and are used to quantify Unknown samples. Internal or external standard curves may be used for data analysis.

- An internal standard curve is generated from Standard samples included in the current run.
- An external standard curve is imported from a previously saved internal standard curve and requires the use of a Calibrator sample.

Available standard curves are displayed in the Standard Curves selection box at the bottom of the Quantification Graph section (Figure 13).

Standard curves are plotted as the best linear fit to two or more unique data points. The slope (m) and intercept (b) of the standard curve are displayed in the Graph section. The correlation coefficient (R), coefficient of determination (R²) and PCR efficiency are automatically calculated and displayed in the lower-left corner of the Graph section.

The graph axes are automatically determined by the maximum and minimum limits of the data and cannot be edited. The Concentration axis is displayed as a Log10 scale and the Threshold scale is displayed as a linear scale.

The standard curve is green in the region of interpolation and red in regions of extrapolation. Points on the standard curve are colour coded as follows:

- Blue filled circles - points used to create an internal standard curve.
- Grey filled circles - replicates used to generate the standard curve.
- Red filled circles - points used to calibrate an external standard curve.
- Open squares - calculated points (i.e., Unknown samples).

6.4.1. Entering Concentrations of Standard Samples

Concentrations may be manually entered for wells containing Standard samples. It is not possible to enter values into this column for wells that contain any other sample type. The concentrations entered will be used in creating or calibrating the currently selected standard curve. To enter Standard sample concentrations:

- Click once on the box in the **"Concen."** column of the Run Data tab and enter a value.

Concentration values will always be displayed in scientific notation rounded to two decimal places (e.g., 1.78E+03). However, you may enter values using standard decimal notation, scientific notation or abbreviated scientific notation. The following are all examples of valid input formats:

Operator Entry	Displayed Value
1.347	1.35E+00
1.347+0	1.35E+00
1.00-04	1.00E-04
1e-4	1.00E-04
100000	1.00E+05
0.00001	1.00E-05

6.4.2. Using an Internal Standard Curve for Quantification

To plot an internal standard curve:

- Click on **“Internal Quantification Analysis”** in the Standard Curves selection box (Figure 13), then select at least two Standard samples with unique concentrations by checking the toggle boxes in the “Incl.” column of the well information section.

If less than two Standards with unique concentrations are included for analysis, the **“Internal Quantification Analysis”** option will be preceded by a red cross and an internal standard curve cannot be generated.

6.4.3. Internal Standard Curves with Replicate Data

If replicates are included in the run (i.e., Standard samples with the same concentration), the Threshold value is calculated as the average of these data. Standard sample concentrations are considered to be equal (replicates) if the displayed values are identical to two decimal point accuracy. An example of three replicate values would be:

Value	Replicate 1	Replicate 2	Replicate 3
Actual	1.246E+01	1.253E+01	12.50
Displayed	1.25E+01	1.25E+01	1.25E+01

6.4.4. Saving an Internal Standard Curve

Internal standard curves can be exported to create an external standard curve. Curves will be saved as .std files, which can be reloaded as external standard curves (see the following section). To save a standard curve:

- Click on **“Save Standard”** in the Standard Curves selection box (Figure 13).

6.4.5. Importing an External Standard Curve

To import an external standard curve from a previously saved .std file:

- Click on **“Load Standards”** in the Standard Curves selection box (Figure 13), then select the appropriate file and click Open. The external standard curve name will appear in the Standard Curves selection box.

Multiple .std files can be selected and imported for each analysis.

6.4.6. Using an External Standard Curve for Quantification

External standard curves require that a Standard sample is used for calibration. Calibrating an external standard curve consists of offsetting the fitted line based on the Calibrator sample, such that a new Y intercept for the line is determined. To plot an external standard curve:

- Click on the appropriate name in the Standard Curves selection box (Figure 13), then select either a single Standard sample, or multiple Standard samples with the same concentration, by checking the toggle box in the **“Incl.”** column of the Well Information section.

Any number of replicate Standard samples with equal concentration can be used as the Calibrator sample.

If two or more standards with unique concentrations are included for analysis, any imported external standard curves will be preceded by a red cross and therefore cannot be generated.


6.5. Editing the Quantification Graph Title

The main graph title defaults to “Quantification Analysis”. To edit this:

- Double-click with the mouse on the graph title and enter the new name into the pop-up dialogue box (Figure 9).

6.6. Displaying Replicate Data on the Quantification Graph

If replicates are included in the run (i.e., Standard samples with the same concentration), the Threshold values displayed on the Quantification graph are calculated as the average of these data. You can choose to display the replicate data on the graph using one of the following options:

- A) Select **“View”** then **“Quantification Replicates”** from the toolbar menu.
- B) Click on the View Replicates in Graph icon  on the toolbar.

7. Using the Melting Curve Tab

7.1. Melting Curve Tab Sections

This tab contains two main sections (Figure 15): Well Information section and Graph section.

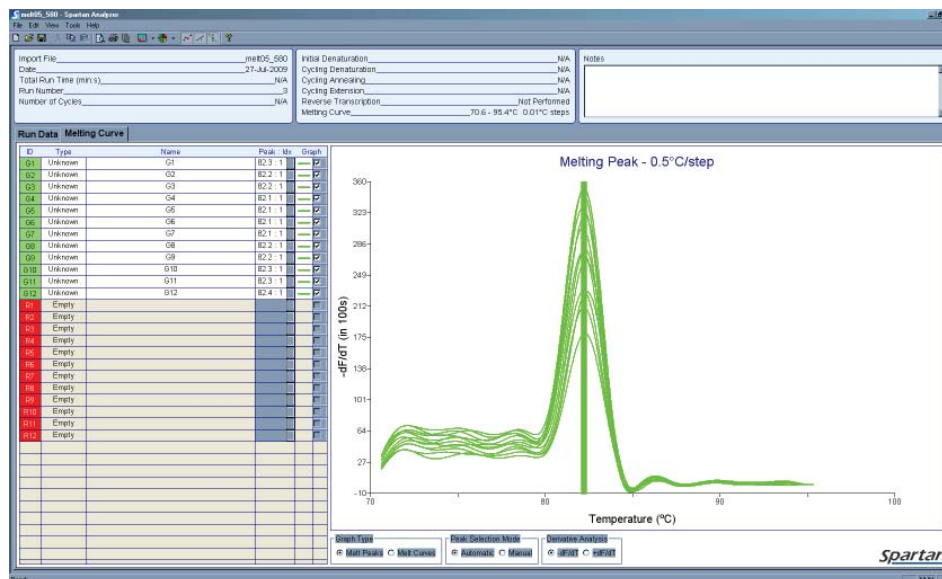


Figure 15. Melting Curve tab

7.1.1. Well Information Section

The Well Information section allows you to specify whether or not to display the data for each well on the adjacent graph, and to view all identified melting peaks (temperatures) for individual wells and channels. Each row in the table corresponds to a specific channel and well on the Spartan DX-12™. The ID column displays the channel (G – Green, R – Red) and well number (1 – 12).

Sample Name and Sample Type cannot be edited in the Well Information section of the Melting Curve tab, because these are linked to the Run Data tab information.

7.1.2. Graph Section

The Graph section of the Melting Curve tab displays data for the wells you have selected (by checking the boxes) in the Well Information section. Melting curve data for green channel wells are plotted using solid lines and melting curve data for red channel wells are plotted using dotted lines. You can choose to display the graph as Melt Peaks or as Melt Curves (Figure 16).

7.2. Choosing the Melting Curve Graph Type

Data in the Melting Curve tab can be displayed as Melt Peaks or as Melt Curves.

The Melt Curves graph plots Temperature (°C) against Fluorescence (arbitrary units (*10³)). The Melt Peaks graph plots Temperature (°C) against the negative or positive first derivative of the Fluorescence (+/- dF/dT (*10²)).

To switch the graphical display between Melt Peaks and Melt Curves:

- Select or unselect the **“Graph Type”** check boxes displayed below the graph.

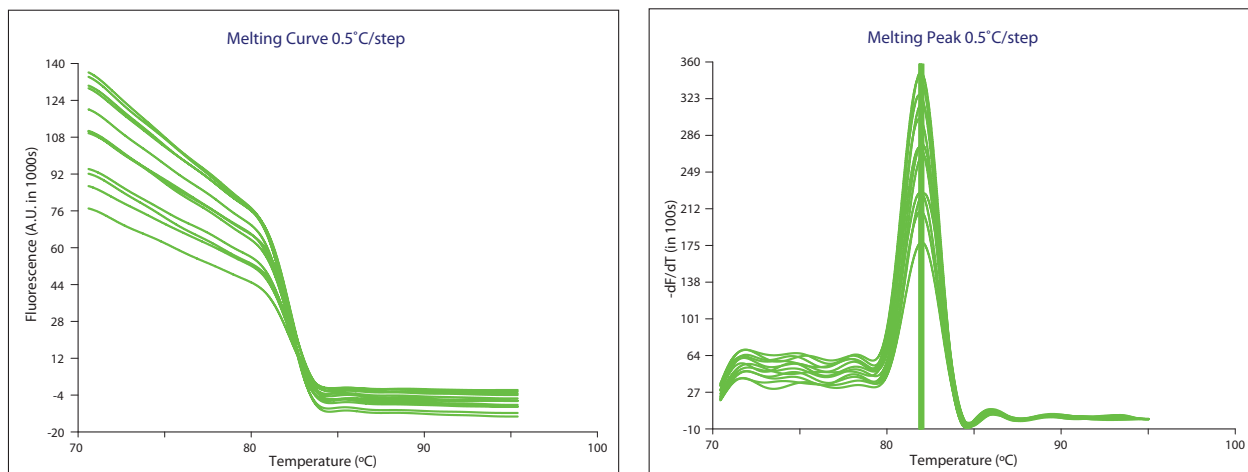


Figure 16. Melt Curves and Melt Peaks data displays in the Melting Curve tab

7.3. Changing Derivative Analysis ($-dF/dT$ or $+dF/dT$)

To switch the graphical display between negative first derivative ($-dF/dT$) and positive first derivative ($+dF/dT$):

- Select or unselect the **“Derivative Analysis”** check boxes displayed below the graph.

This option is only applicable when the Melting Curve graph type is set to **“Melt Peaks”**. Toggling between these options when the graph type is set to **“Melt Curves”** will have no influence on the graphical display.

7.4. Changing Data Displayed on the Melting Curve/Peak Graph

To change which data (i.e., which wells) are displayed on the graph:

- Select or unselect the check boxes for each well (in the **“Graph”** column of the well information section).

To quickly change the data displayed the following functions may be used:

- CTRL + mouse click on a single box will uncheck all other boxes; or
- SHIFT + mouse click on any box will select all boxes.

7.5. Editing the Melting Curve/Peak Graph Title

The main graph title defaults to Melting Curve or Melting Peak, depending on the type of graphical display you have selected (see section 7.2). To edit the graph title:

- Double-click with the mouse on the text and enter the new name into the dialogue box (Figure 9).

7.6. Changing the Melting Curve/Peak Graph Axes Scales

7.6.1. Fluorescence (or $-df/dT$) axis

The default minimum limit, maximum limit and tick intervals of the Fluorescence axis are automatically determined by the extreme values of the data displayed. These settings can be manually adjusted using one of the following options:

- Right mouse-click on the axis and select **“Edit Scale”** from the menu; or

- B) Double click on the axis, then select **“Manual Scale”** and enter values into the pop-up dialogue box (Figure 11). Selecting **“Automatic Scale”** will adjust settings to the default values.

7.6.2. Temperature Axis

The Temperature axis scale is automatically determined by the data available in the imported data file and cannot be edited.

7.7. Changing the Peak Selection Mode

When the graph type is set to **“Melt Peaks”**, you have two options available for peak selection: Automatic and Manual.

- In **“Automatic mode”**, the software automatically identifies melting peaks for each well included in the display (by checking the toggle boxes for each well – see section 7.4).
- In **“Manual mode”**, you may manually insert, remove, and reposition melting peaks using the mouse.

Melting peaks are represented on the graph by vertical lines.

In both Automatic and Manual modes, if a single well is selected for display, the temperature values associated with each melt peak for that well are displayed below the Temperature axis in the Graph section.

7.8. Manually Entering Melt Peaks

To manually define melt peaks in Manual Peak Selection mode, you must select a single well for display in the Graph section (by checking the toggle boxes for each well – see section 7.4). You may define multiple melt peaks for each well.

To define melt peaks manually, use the following functions:

- Double-click with the mouse on **“an area of the graph”** to create a melt peak at that position.
- Double-click with the mouse on **“an existing vertical line”** to remove that peak.
- Drag-and-drop an existing melt peak line with the mouse operations to change the peak value.

7.9. Modifying Automatic Melting Peak Selection Parameters

In Automatic Peak Selection mode you can modify the parameters used to determine melt peaks, using one of the following options:

- A) Select **“Edit”** then **“Melting Peak Analysis”** from the toolbar menu; or
B) Use the Hotkey <Ctrl + M>.

Enter values into the pop-up dialogue box (Figure 17). The parameters that can be altered are:

- Minimum Peak Separation (°C) – this value describes the minimum temperature difference that must be present between individual defined melting peaks.
- Relative Minimum Peak Amplitude (in 100s) – this value describes the minimum $-dF/dT$

or +dF/dT value, relative to background values at either side of the peak, that must be reached for a melt peak to be defined.

- Absolute Minimum Peak Amplitude (in 100s) – this value describes the absolute minimum –dF/dT or +dF/dT value that must be reached for a melt peak to be defined.

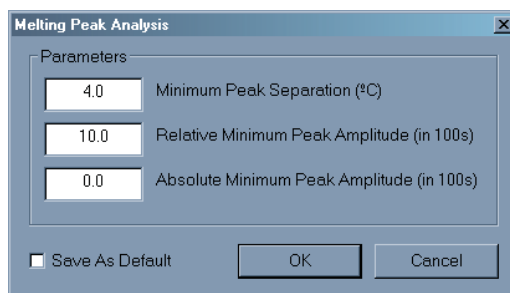


Figure 17. Melting Peak Analysis dialogue box

To save the current settings as default, check the **“Save As Default”** box and press **“OK”**.

7.10. Viewing Peak : Idx Melting Profiles

The Melting Peak temperature profiles for each well are displayed in the Peak : Idx column of the Well Information section in the Melting Curve tab. To view the melting peak profiles for individual wells:

- Click with the mouse on the **“grey box to the right side of the Peak : Idx column”** in the Well Information section

The melting peak profiles are displayed in the format:

Melting Peak Temperature (°C) : Index (primary peak = 1, secondary peak = 2, etc.).

For example, a profile with two defined melting peaks would be displayed as:

84.0 : 1

79.5 : 2

The software maintains two separate melting peak lists for the Automatic and Manual Peak Selection modes. The values displayed in the list are therefore determined by your chosen Peak Selection mode (see section 7.7). In Manual Peak Selection mode the list will display the eight most recently selected peaks.

8. Well Information Templates


A well information template is used to define the Sample Type, Sample Name, and Graphing Colour for each well and channel. Templates can be created and saved in advance, then applied to imported data in Spartan Graphing & Analysis software.

Examples of situations where you may find well information templates useful are:

- You are performing multiple identical runs in the Spartan DX-12™; or
- You always position your Positive control(s) and/or Negative control(s) and/or Unknown samples in the same wells; or
- You have colour preferences for graphing particular channels and/or wells.

8.1. Creating or Editing Existing Well Information Templates

To edit an existing or create a new well information template, open the Edit Well Information Templates dialogue box (Figure 18) by using one of the following options:

- A) Select **"Edit"** then **"Well Templates"** from the toolbar menu; or
- B) Click on the Edit Well Information Templates icon  on the toolbar; or
- C) Use the Hotkey <Ctrl + T>.

To create a new template, click **"Add"**. An additional name will appear in the Available Templates list. This name can be edited by typing into the **"Selected Template"** text box. Click **"OK"** to save the new template. Once saved, the new template name will appear in the Templates list.

Note – New templates can be locked when in Administrator mode (see Section 10).

To modify an existing template, select the appropriate name from the available Templates list. The information related to the selected template will then be displayed.

Note - Locked templates can only be modified when in Administrator mode (see Section 10).

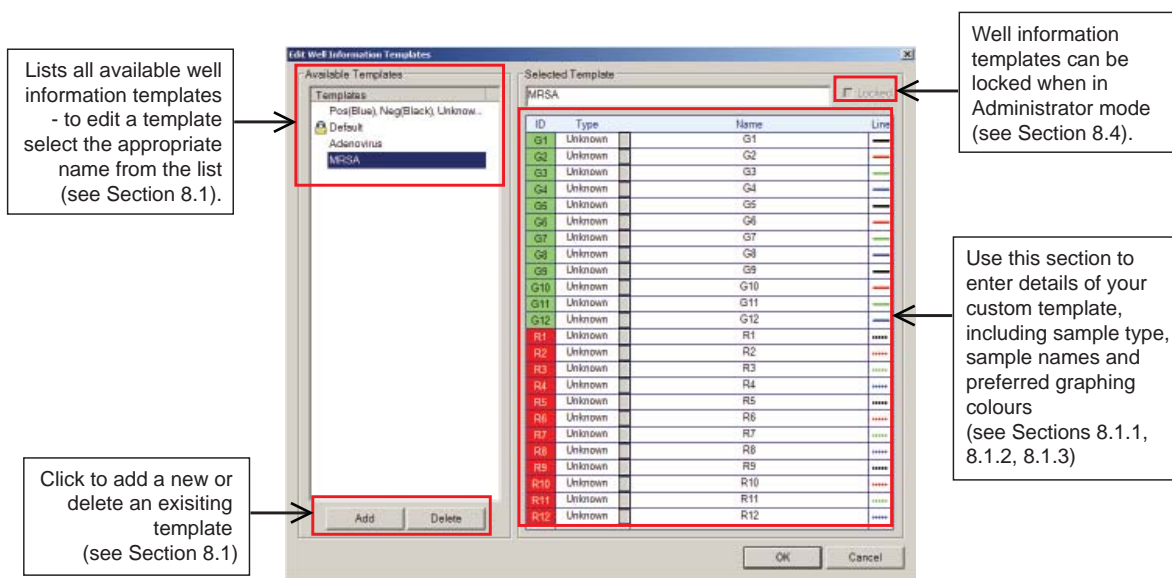


Figure 18. Edit Well Information Templates dialogue box

8.1.1. Defining Sample Names

The sample names associated with each well and channel default to the well ID (G1, G2, etc. or R1, R2, etc.). To edit these:

- Click once on the sample name (displayed in the Name column) and type the new name.
- You may enter a name up to 60 characters in length. When the name field is not wide enough to display the entire name, the complete name may be displayed as a tool tip when the mouse is floated over the field.

8.1.2. Defining Sample Types

The sample types for each well and channel can be specified as Positive, Negative, Standard or Unknown. See Section 5.3 for detailed descriptions of these sample types. To specify the sample type for each well and channel, use one of the following options:

- A) Click once on the **“sample type”** (displayed in the **“Type”** column), then use the hot keys displayed in Section 5.3 to set the sample type; or
- B) Click on the drop-down selection button to the right side of the **“Type”** column.


8.1.3. Defining Graphing Colours

To change the graphing colour for each well and channel:

- Click once on the coloured line in the **“Line”** column, then use the pop-up dialogue box (Figure 8, Section 5.6) to define your colour preferences.

8.2. Saving Current Settings as a Well Information Template

When a data file is imported into Spartan Graphing & Analysis software, the current settings (Sample Name, Sample Type, Graphing Colour) may be saved as a well information template. To do this:

- Click on the drop-down menu adjacent to the Well Information Templates icon  on the toolbar, then select **“Save Well Information”**.

The Save As Well Template dialogue box will appear (Figure 19) allowing you to enter a new template name. If a pre-existing template is selected from the **“Current Templates”** list, this template will be updated with the new settings.

8.3. Deleting a Well Information Template

To delete an existing template, open the Edit Well Information Templates dialogue box (see Section 8.1), then select the template name from the list and click **“Delete”**.

Note - Locked templates can only be deleted when in Administrator mode (see Section 10).

8.4. Locked Well Information Templates

Well information templates can be locked when in Administrator mode (see Section 10). Locked templates are indicated by a padlock graphic to the left of the template in the list. The locked checkbox to the right of the template name will only be available when an administrator is logged into the system.

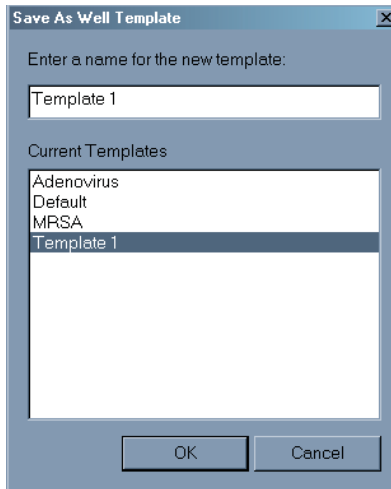



Figure 19. Save As Well Template dialogue box

8.5. Applying a Well Information Template to Current Data

To apply a well information template to current data:

- Click on the drop down menu adjacent to the Well Information Templates icon “” on the toolbar menu, then select the appropriate template name.

The drop down menu displays the five most recently selected templates. Additional saved templates can be accessed by selecting “**More Templates**” from the drop down menu.

9. Calling Results Definitions

9.1. Overview of the Calling Results Feature

The Calling Results feature of Spartan Graphing & Analysis software provides a convenient way to speed up and simplify Real-Time and End-Point data analyses, by enabling automatic examination of your results by the software. This feature substantially reduces or eliminates the need for user interpretation of data, allowing you to quickly assess if your unknown samples contain a target gene of interest. Using the Calling Results feature, it is possible to create and save your own custom data interpretation scenarios and apply them as templates to PCR and Melt Curve data.

Based on a user-defined Calling Results Definition, the software uses a set of algorithms for automated results calling of the following sample types:

Sample Types	Possible Outcomes
Positive Controls	PASS FAIL
Negative Controls	PASS FAIL
Unknowns	MINUS PLUS PLUS <i>target name</i> INC (inconclusive)

A Calling Results Definition refers to a set of user-defined criteria that is used to assess run data imported into the software. Criteria refer to the conditions used to assess data for each channel and each well. These include:

- Ct - must be \geq or \leq a set value (Real-Time data analysis only).
- Δ FI - must be \geq or \leq a set value (End-Point data analysis only).
- Melt Curve peak - must be \geq or/and \leq a set value.

For each well, criteria can be entered in the Calling Results Definition dialogue box (Figure 20) for targets in both green and red channels. In addition, as successful amplification of an internal positive control (IPC) is often used in multiplex PCR reactions to clarify that reactions do not contain PCR inhibitors, the Calling Results feature also allows you to define criteria for an IPC in each well.

Result calls are made based on an assessment of run data against:

- 1) Your user-defined non-IPC Criteria (i.e., criteria set for non-IPC targets in the green and red channels)
AND (if required)
- 2) Your user-defined IPC Criteria (i.e., criteria set for the IPC target).

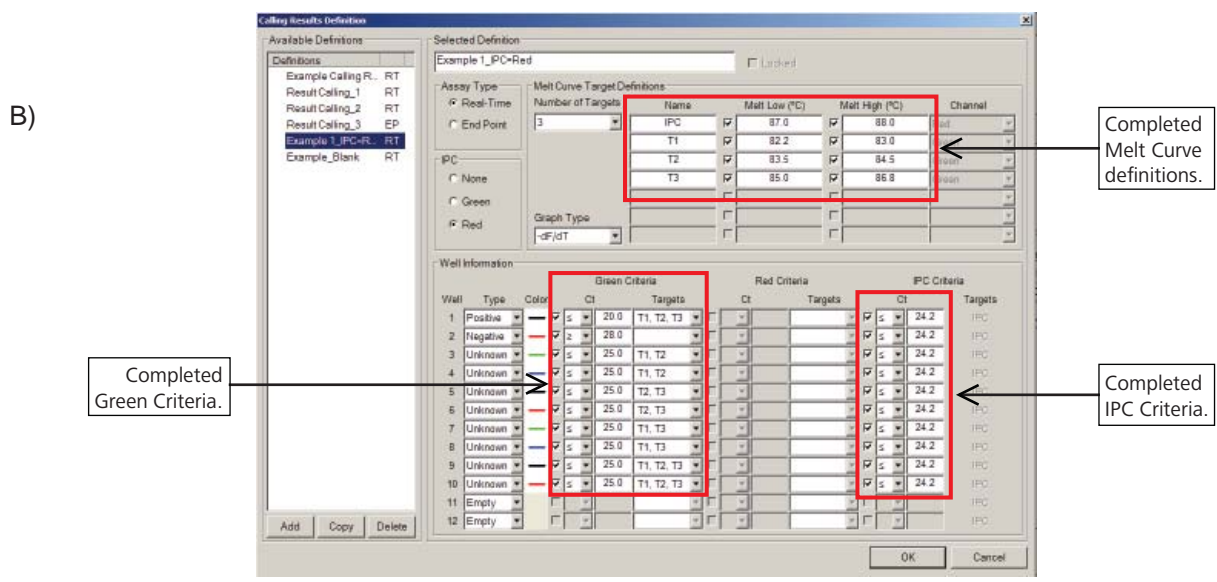
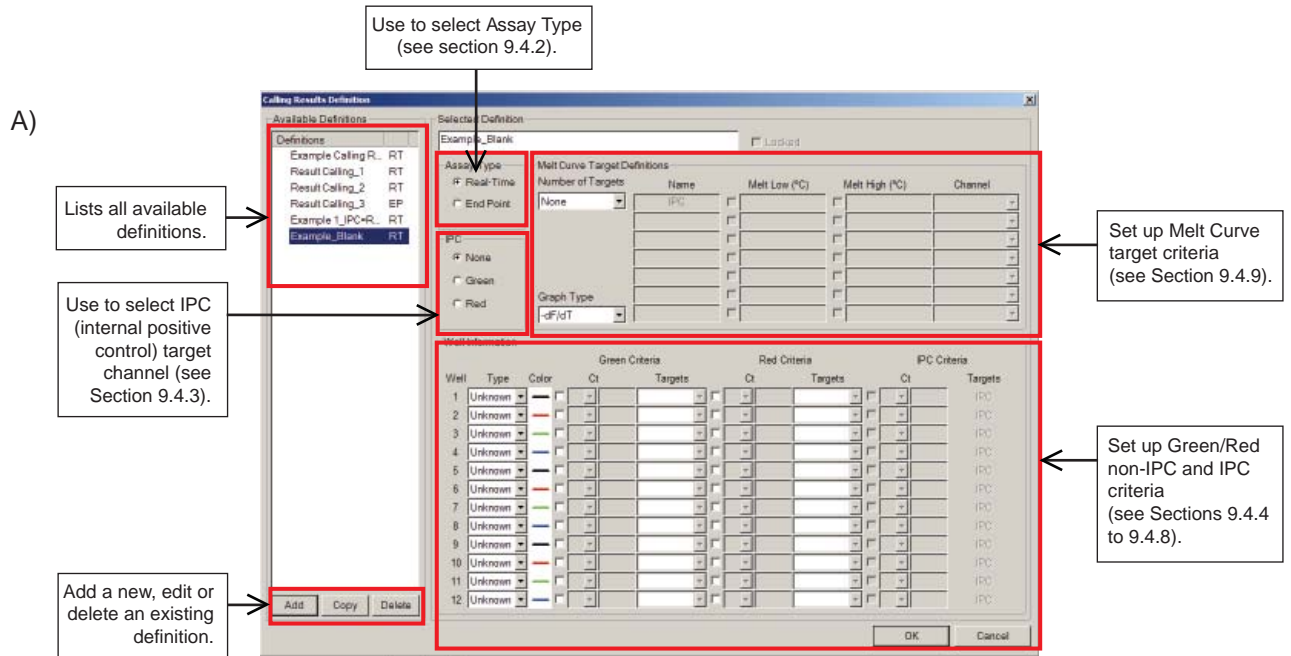


Figure 20. Examples of Calling Results Definition dialogue box. A) Empty Calling Results Definition dialogue box. B) Completed Calling Results Definition dialogue box.

9.2. Calling Results Outcomes of Positive and Negative Control Samples

The software will call each Green and Red well containing a Positive or Negative control sample as PASS or FAIL, depending on whether the data meets or fails to meet the non-IPC or IPC criteria for that well in the Calling Results Definition. The table below summarizes Calling Results outcomes of Positive and Negative control sample wells:

If the Channel of the well being assessed is the same as the channel of the IPC:	
IPC Criteria met?	Calling Results Outcomes
<input checked="" type="checkbox"/>	PASS
<input checked="" type="checkbox"/>	FAIL

If the Channel of the well being assessed is different from the channel of the IPC:	
non-IPC Criteria met (Green and Red)?	Calling Results Outcomes
<input checked="" type="checkbox"/>	PASS
<input checked="" type="checkbox"/>	FAIL

Figure 21 (on page 32) outlines the procedure flow used by the software to call results of Positive and Negative control samples.

9.3. Calling Results Outcomes of Unknown Samples

The software will call each Green or Red well containing an Unknown sample as PLUS, PLUS-target name, MINUS or INC.

When using a Calling Results definition, it is not possible to define IPC criteria and non-IPC criteria in the same channel (see Section 9.4.3). For example, if the channel of the IPC is set to Red, all non-IPC targets must be in the green channel. If the channel of the IPC is set to Green, all non-IPC targets must be in the red channel. Therefore, a result will not be called (i.e., well outcome will be left blank) when the channel (green or red) of the well being assessed is the same as the channel set for the IPC (as selected by the user in the Calling Results Definition).

If Positive and Negative control samples are included in the Calling Results Definition, the Calling Results outcome of Unknown samples will be dependent on all Positive and Negative controls meeting their own set criteria. If one or more of the Positive or Negative control samples are called as FAIL, all Unknown samples will be called as INC. If IPC criteria are also included in the Calling Results Definition for an unknown well, the Calling Results outcome will additionally depend on whether these are met. If the IPC criteria are not met for an Unknown sample well, the Calling Results outcome for that well will be INC.

If all Positive / Negative controls are called as PASS, and the IPC criteria set for the well are met, the Unknown sample will be called as MINUS, PLUS or PLUS-target name. In this case, the Calling Results outcome will depend on the green/red non-IPC criteria set for the well. If the green/red non-IPC criteria are not met, the sample will be called as MINUS. If the green/red non-IPC criteria are met the sample will be called as PLUS. If these criteria also include one or more Melt Curve Target Definitions which are met, the outcome will be PLUS-target name(s). The target names can be entered by the user in the Calling Results Definitions dialogue box (Figure 20), (see Section 9.4.9).

POSITIVES AND NEGATIVE CONTROLS

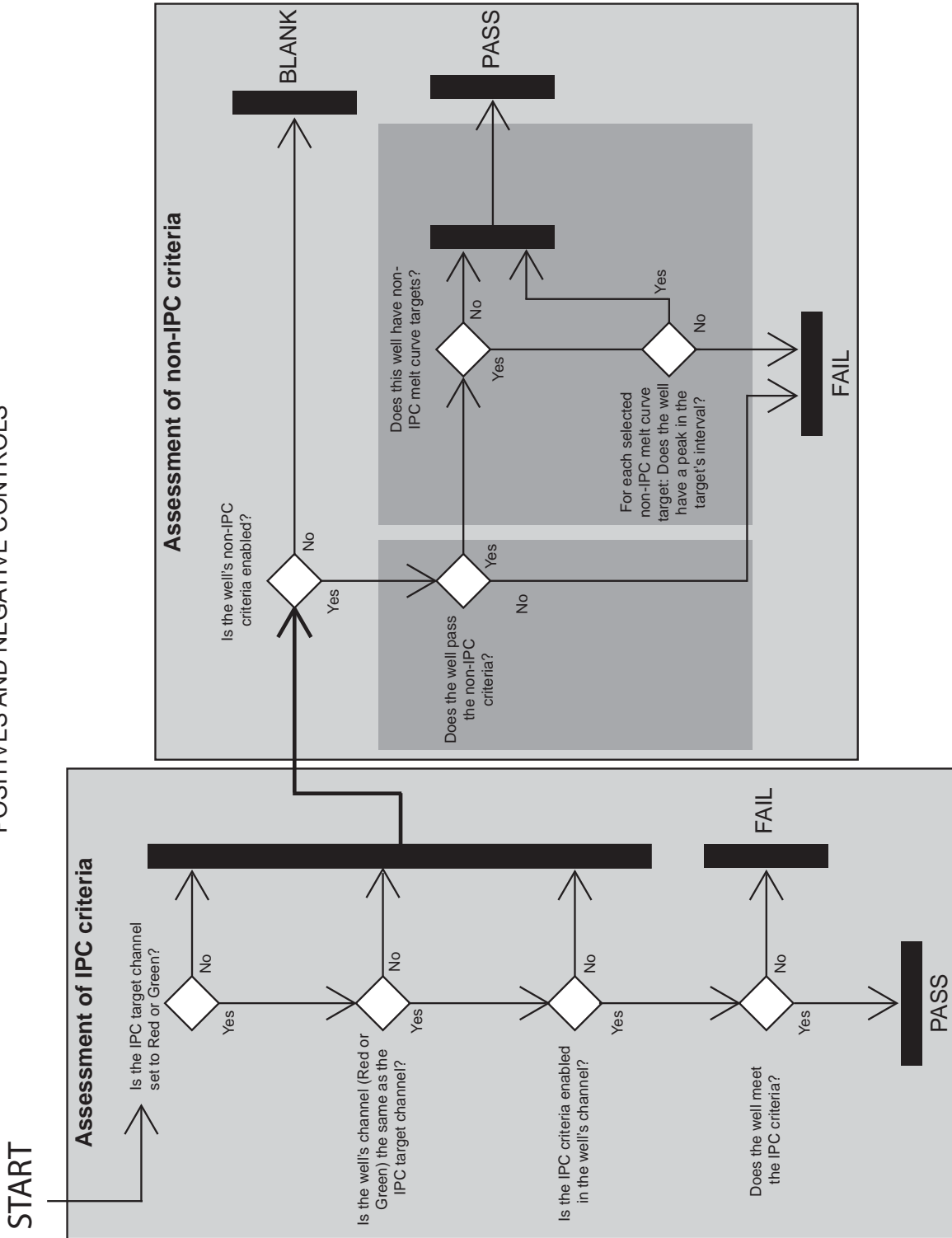


Figure 21. Procedure used to call Positive and Negative control samples

The table below summarizes Calling Results outcomes of Unknown sample wells:

If the channel of the well being assessed is <u>different</u> from the channel of the IPC:			
All Positive / Negative Controls PASS?	IPC Criteria (in the corresponding well of the other channel) met or N/A?	Non-IPC Criteria (Green and/or Red) met?	Calling Results Outcomes
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	PLUS or PLUS <i>target name</i>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	MINUS
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC

If the channel of the well being assessed is the <u>same as</u> the channel of the IPC:		
All Positive/Negative Controls PASS?	IPC Criteria met?	Calling Results Outcomes
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	INC
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	BLANK
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	BLANK



Figure 22 (on page 34) outlines the procedure flow used by the software to call Unknown samples.

9.4. Setting Up Criteria for a Calling Results Definition

The following paragraphs explain how to use each section of the Calling Results Definitions dialogue box when setting up a new Definition.

9.4.1. Opening the Calling Results Definition dialogue box

To edit an existing or create a new Calling Results Definition, use one of the following options to open the Calling Results Definition dialogue box (Figure 20):

- A) Select **"Edit"** then **"Calling Results"** from the toolbar menu; or
- B) Click on the Edit Calling Results Definitions icon ""; or
- C) Click on the drop down menu adjacent to the Edit Calling Results Definitions icon on the toolbar "", then select **"Edit Definitions"**; or
- D) Use the hotkey <Ctrl + R>.

To edit an existing Definition, select the appropriate name from the **"Available Definitions"**

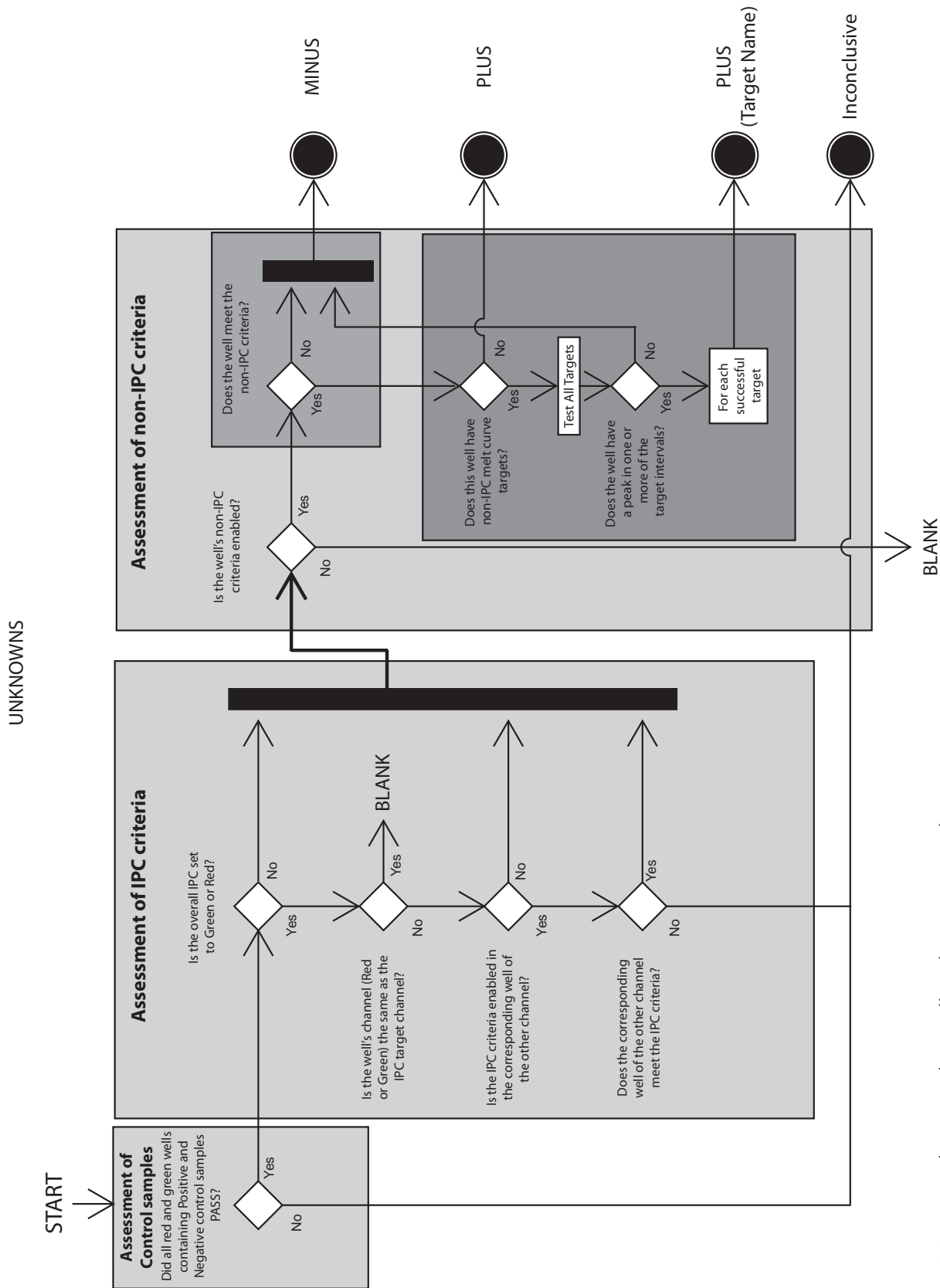


Figure 22. Procedure used to call Unknown samples

list. Details of the selected Definition will then be displayed. To save any changes, click **“OK”**.

To create a new Definition, click **“Add”**. An additional name will appear in the Available Definitions list. The name can be edited by selecting the new Definition from the list and typing into the **“Selected Definition”** text box. To save the new Definition, click **“OK”**.

Note: Definitions can be locked and unlocked when in Administrator mode (see Section 10). Existing locked Definitions can only be modified by an Administrator.

9.4.2. Defining the Assay Type

Calling Results Definitions can be set up for analyses of either Real-Time data or End-Point data. To select the assay type to which the Definition will be applied, click the appropriate check-box in the Assay Type section of the Calling Results Definitions dialogue box (Figure 23).

If the assay type is selected as **“Real-Time”**, Green and Red criteria may be entered as \geq or \leq a desired Ct value. If **“End-Point”** is selected, these criteria may be defined as \geq or \leq a desired Δ FI (fluorescence at last cycle – fluorescence at first cycle).

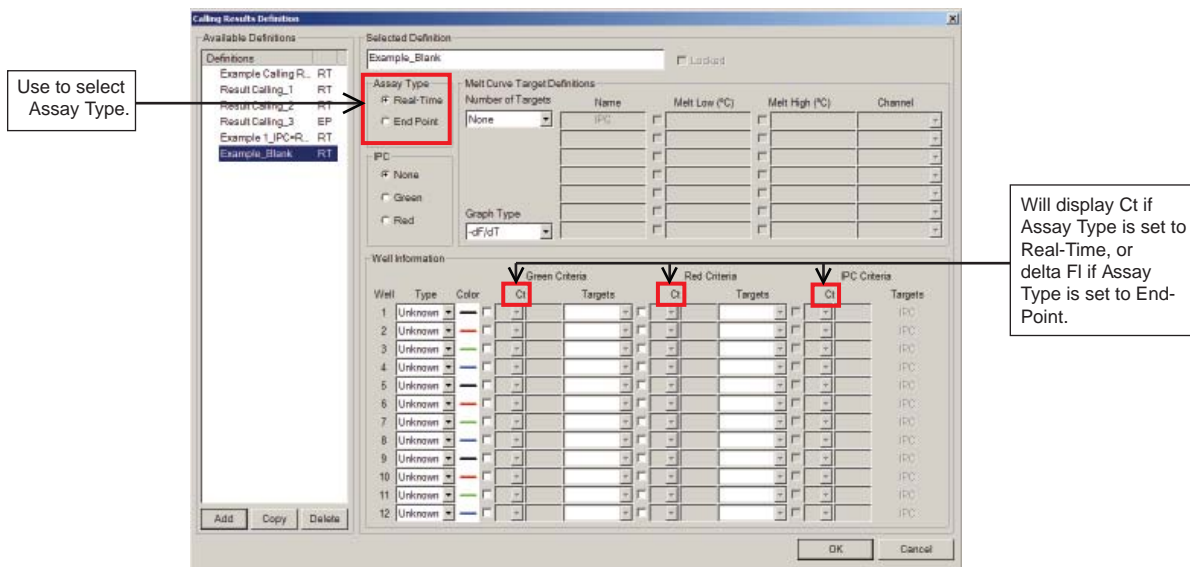


Figure 23. Assay Type section of the Calling Results Definitions dialogue box

9.4.3. Defining the IPC (Internal Positive Control) Channel

Successful amplification of an internal positive control (IPC) can be used in multiplex PCR reactions to clarify that reactions do not contain PCR inhibitors. When setting up a Calling Results Definition, you have the option to include or exclude analysis of IPC data by clicking the appropriate check-box in the **“IPC”** section of the Calling Results Definitions dialogue box (Figure 24).

If you do not wish to include analysis of IPC data, toggle the check-box to **“None”**. In this case, you will not be able to enter any IPC Criteria (i.e., the corresponding section of the Calling Results Definition dialogue box will be grayed out / unavailable for editing). If you do wish to use IPC data for results calling, toggle the check-box to **“Green”** or **“Red”**. The IPC can only be selected in one channel.

Note that it is not possible to define IPC criteria and non-IPC criteria in the same channel. For example, if the channel of the IPC is set to **“Red”**, all non-IPC targets must be in the green

channel. In this case, the Red Criteria section of the Calling Results Definition dialogue box will be grayed out / unavailable for editing. If the channel of the IPC is set to **“Green”**, all non-IPC targets must be in the red channel and the Green Criteria section of the Calling Results Definition dialogue box will be grayed out / unavailable for editing.

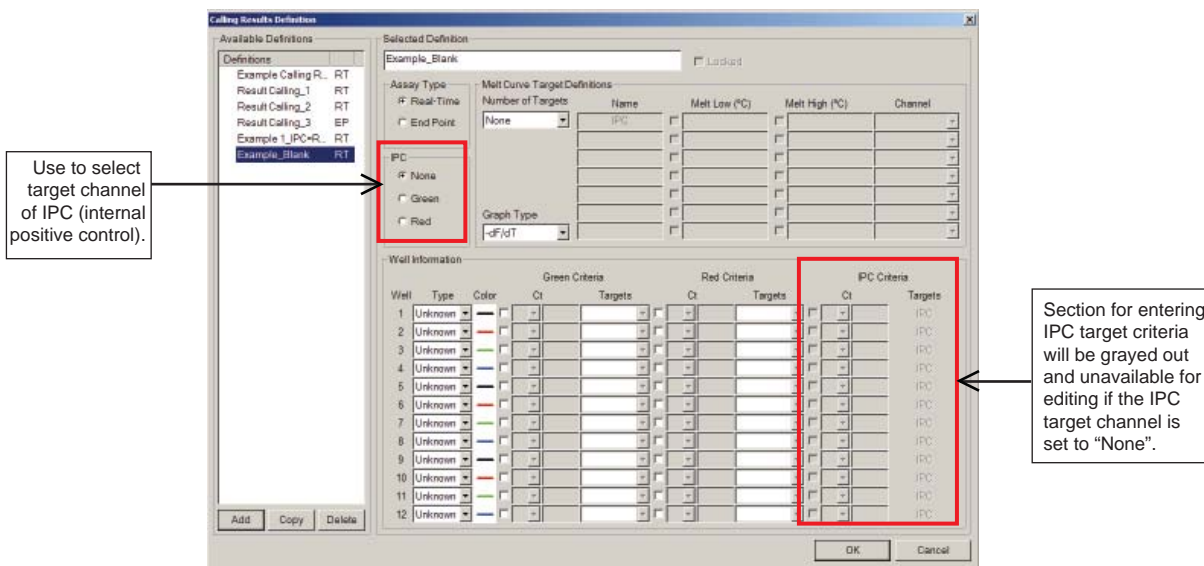


Figure 24. IPC section of the Calling Results Definitions dialogue box

9.4.4. Defining Sample Types

The sample types for each well can be specified as **“Positive”**, **“Negative”**, **“Unknown”** or **“Empty”** by clicking on the drop down selection button to the right side of the **“Type”** column (Figure 25). A well should be set to **“Empty”** if you do not plan to include a sample in the well when performing a run on the Spartan DX-12™.

Following application of a Calling Results Definition to run data imported into the software (see Section 9.5 – Applying a Calling Results Definition to current data), wells which were set to **“Unknown”** in the Definition can be changed to **“Empty”** in the Run Data tab. Wells which were set to **“Positive”**, **“Negative”** or **“Empty”** in the Definition can not be changed in the Run Data tab after the Definition is applied to the data.

9.4.5. Defining Graphing Colors

To change the graphing color for each well:

- Click once on the colored line in the **“Color”** column (Figure 25), then use the pop-up dialogue box (Figure 8, Section 5.6) to define your color preferences.

Note that when the Calling Results Definition is applied to data in the software, both green and red channel wells will be displayed using the same color – data for green channel wells will be plotted using solid lines and data for red channel wells will be plotted using dotted lines.

9.4.6. Defining Green Channel Criteria (Non-IPC Criteria)

To include Green channel criteria for a well, select the check box on the left hand side of the **“Green Criteria”** section of the Calling Results Definitions dialogue box (Figure 25). When this box is checked you will be able to enter both Ct / Δ FI criteria and Melt Curve targets for the well.

To define the Ct (if Assay Type is set to Real-Time), or Δ Ft (if Assay Type is set to End-Point) criteria:

- Select “ \geq ” or “ \leq ” from the drop-down menu, then enter your desired value (up to one decimal place).

To define Melt Curve Targets for the well:

- Select the appropriate target name(s) from the drop-down menu in the “**Targets**” column.

The target names available for selection in the drop down menu are dependent on the target names entered in the Melt Curve Targets Definitions section of the dialogue box (see Section 9.4.9). Note that only targets that are in the green channel will be available for selection from the drop down menu when defining Green criteria. For example, if you enter the target name STRAIN A in the Melt Curve Targets Definitions section, and the channel for this target is set to Green, the target name STRAIN A will be available for selection in the drop down menu. If you enter the target name STRAIN B in the Melt Curve Targets Definitions section, and the channel for this target is set to Red, the target name STRAIN B will not be available for selection in the drop down menu.

Note that you will only be able to enter Green criteria for each well if you have set the IPC channel to “**Red**” or “**None**” (see Section 9.4.3). If the IPC channel is set to “**Green**”, the “**Green Criteria**” section of the dialogue box will be grayed out / unavailable for editing.

Use to specify your preferred graphing color for each line (see Section 9.4.5).

Use the drop-down menu to select the sample type in each well (see Section 9.4.4).

Enter your Green/Red non-IPC target criteria here (see Sections 9.4.6 and 9.4.7). If the IPC target channel is set to “Green” the Red Criteria section will be grayed out. If the IPC target channel is set to “Red”, the Green Criteria section will be grayed out.

Enter your IPC target criteria here (see Section 9.4.8). This section will be grayed out and unavailable for editing if the IPC target channel is set to “None”.

Figure 25. Well information section of the Calling Results Definitions dialogue box

9.4.7. Defining Red channel criteria (non-IPC criteria)

The procedure for defining Red channel criteria for each well follows the same principle as the procedure for defining Green channel criteria (see Section 9.4.6).

Note that you will only be able to enter Red criteria for each well if you have set the IPC channel to “**Green**” or “**None**” (see Section 9.4.3). If the IPC channel is set to “**Red**”, the Red Criteria section of the dialogue box will be grayed out / unavailable for editing.

9.4.8. Defining IPC criteria

The procedure for defining IPC criteria for each well follows the same principle as the procedure for defining Green channel criteria (see Section 9.4.6).

Note that you will only be able to enter IPC criteria for each well if you have set the IPC channel to **“Red”** or **“Green”** (see Section 9.4.3). If the IPC channel is set to **“None”**, the **“IPC Criteria”** section of the dialogue box will be grayed out / unavailable for editing.

9.4.9. Melt Curve Targets Definitions

This section allows you to enter melt peak criteria for the IPC and up to six additional targets. To select the number of targets you wish to include, use the **“Number of Targets”** drop down menu (Figure 26). If the Number of Targets is set to **“None”** you will not be able to enter any melt peak criteria (i.e., the corresponding section of the dialogue box will be grayed out / unavailable for editing). In addition, if the IPC channel is set to **“None”** (see Section 9.4.3) you will not be able to enter any melt peak criteria for the IPC (i.e., the corresponding line of the dialogue box will be grayed out / unavailable for editing).

For each target you may enter your own custom target names (up to 10 characters) in the **“Name”** column of the Melt Curves Target Definitions section (Figure 26). Names will appear in the **“Targets”** drop down menu for each well in the well information section (see Sections 9.4.6 & 9.4.7).

You have the option to define the following melt peak criteria for each target:

- Melt Low (°C) only – the software will look for a melt peak greater than or equal to the value entered.
- Melt High (°C) only – the software will look for a melt peak less than or equal to the value entered.
- Melt Low (°C) and Melt High (°C) – the software will look for a melt peak between the Low and High values entered.
- Channel – the software will look for a melt peak in the selected channel (Green or Red).

To enter Melt Low and/or Melt High criteria for a target:

- Select the check boxes on the left hand side of the **“Melt Low (°C)”** and/or **“Melt High (°C)”** sections of the Calling Results Definitions dialogue box (Figure 26), then enter your desired values (values can be entered up to one decimal place).

Since it is not possible to define IPC criteria and non-IPC criteria in the same channel (see Section 9.4.3), the channels available for selection for each melt peak target are dependent on the IPC channel setting. For example, if the IPC channel is set to **“None”**, melt peak target channels can be set to **“Green”** or **“Red”**. If the IPC channel is set to **“Red”**, melt peak target channels can be set to **“Green”** only. If the IPC channel is set to **“Green”**, melt peak target channels can be set to **“Red”** only.

To select the channel (Green or Red) for each target:

- Select the appropriate channel from the drop down menu in the **“Channel”** column (Figure 26).

The **“Graph Type”** can be defined as $-dF/dT$ or $+dF/dT$ (negative or positive first derivative) using the drop down menu in the Calling Results Definitions dialogue box (Figure 26). This refers to the derivative type you prefer to use when displaying Melt Peak graphs in the Melting Curve tab of the software (see Section 7.3). If the **“Number of Targets”** is set to **“None”** this setting will be ignored by the software.

The screenshot shows the 'Calling Results Definitions' dialog box. It has several sections: 'Available Definitions' on the left, 'Selected Definition' at the top, 'Melt Curve Target Definitions' in the middle, and 'Well Information' at the bottom. The 'Melt Curve Target Definitions' section contains a table with columns for 'Number of Targets', 'Name', 'Well Low (°C)', 'Well High (°C)', and 'Channel'. The 'Well Information' section contains a table with columns for 'Well', 'Type', 'Color', 'Ct', 'Targets', and 'IPC Criteria'. Callouts point to various fields: 'Number of Targets' (set to 'None'), 'Name' (empty), 'Graph Type' (set to '-dF/dT'), 'Channel' (dropdown menu), and the 'Targets' and 'IPC Criteria' columns in the 'Well Information' table.

Use the drop-down menu to select the number of non-IPC targets you would like to define melt curve criteria for. A maximum of 6 targets may be selected. If the number of targets is set to “None”, the remainder of the Melt Curves Target Definitions section will be grayed out.

Use the drop-down menu to specify the graphing type of melt curve data to which the definition will be applied. Graph Type can be selected as negative or positive first derivative.

Enter your own custom non-IPC target names here (up to 10 characters). Names entered will appear in the drop-down menu for each well in the “Targets” columns of the Well Information section.


Use the drop-down menu to select the channel of each target. If the IPC channel is set to “Green”, the drop-down menu will only display Red. If the IPC channel is set to “Red”, the drop-down menu will only display Green.

Enter your melt peak criteria for each target here. You may enter a low temperature cutoff value only, a high temperature cutoff value only, or both.

Figure 26. Melt Curve Targets Definitions section of the Calling Results Definitions dialogue box

9.5. Applying a Calling Results Definition to Current Data


To apply a Calling Results Definition to current data:

- Click on the drop down menu adjacent to the Edit Calling Results Definitions icon  on the toolbar menu, then select the appropriate Definition name.

The drop down menu displays the five most recent Definitions. Additional saved Definitions can be accessed by selecting **“More Definitions”** from the drop down menu.

9.6. Removing a Calling Results Definition From Current Data

To remove a previously applied Definition from current data and view data without the Calling Results interpretation:

- Click on the drop down menu adjacent to the Well Information Templates icon  on the toolbar menu, then select the appropriate template name.

10. Administrator Mode

Administrator mode enables access to various protected features. An administrator has the ability to lock or unlock existing Well Information Templates and Calling Results Definitions, and to delete or modify locked templates or definitions (see Sections 8 & 9). Locking means that non-administrators cannot alter any information in the template or delete the template.

10.1. Accessing Administrator Mode

To log in or log out of Spartan Graphing & Analysis software as an Administrator:

- Select **"File"** then **"Administrator Mode"** from the toolbar menu.

A password is required for access to Administrator mode, the default is set to (case-sensitive): a1b2c3d4. To change the default password:

- Select **"File"** then **"Change Password"** from the toolbar menu.