

Some new terms

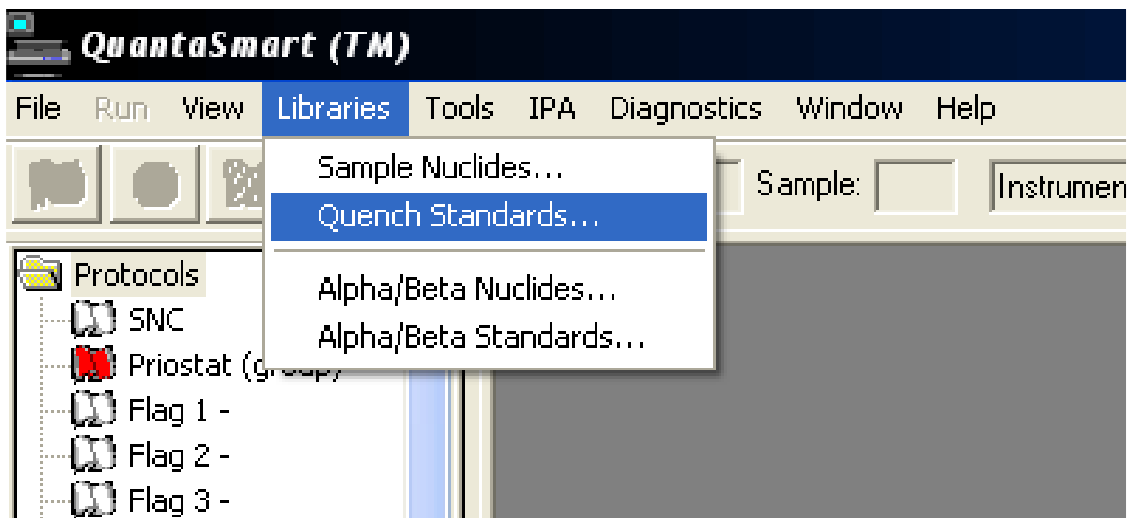
- Assay is now used instead of Protocol.
- The Protocol flag is still used but is called a Flag in the software.
- Quench curves are no longer specific to a protocol.
- There are some actions that should be done before you set up and assay.
- Please note that there is Hypertext help in QuantaSmart. When you are in a page and you click on HELP you will see Help displayed for that particular page. If you position the cursor over the part you are concerned with and then click the mouse it will display the options available for that part.

Before you start

Actions that should be done before an assay.

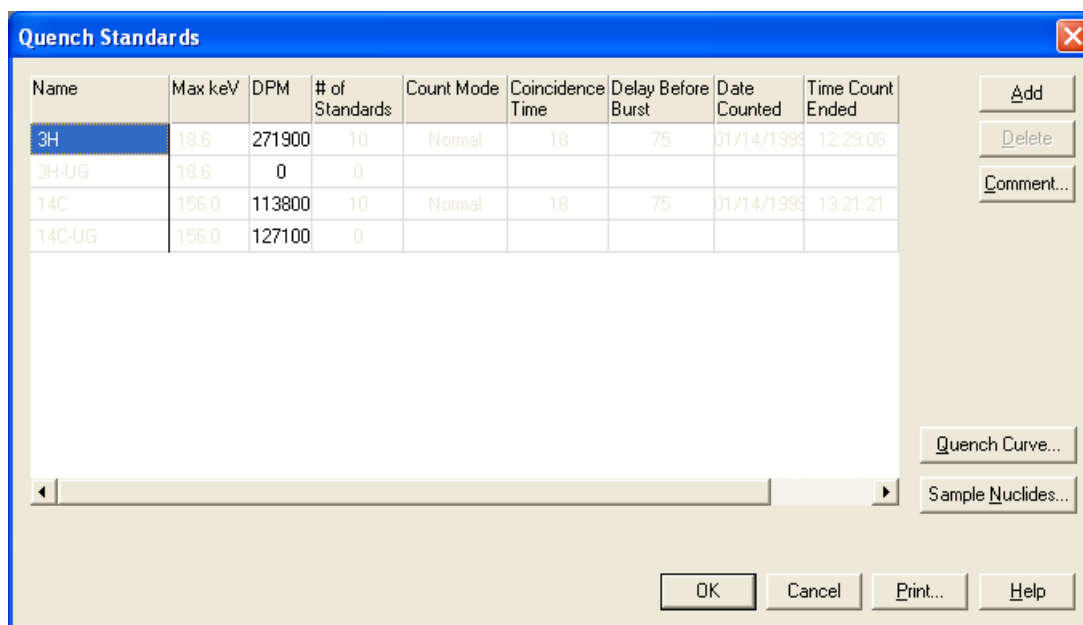
Quench Standards

Identify the quench curve that you will need for your assay. If the quench curve is not there then you will need to load it. The quench standards page is found on the QuantaSmart desktop



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The page will look something like this



The quench standards that are available will be displayed. The quench standards will show the number of standards counted and the activity in the standards and the Max keV. The quench standards must have all of the above or you will not be able to use it.

There are factory set quench standards that will be suitable for most applications. These are greyed out and labelled 3H, 3H-UG, 14C and 14C-UG. The UG means that they may be used with any high flash point cocktail such as GoldStar or ProSafe that use DIN as their solvent.

Sample Nuclides

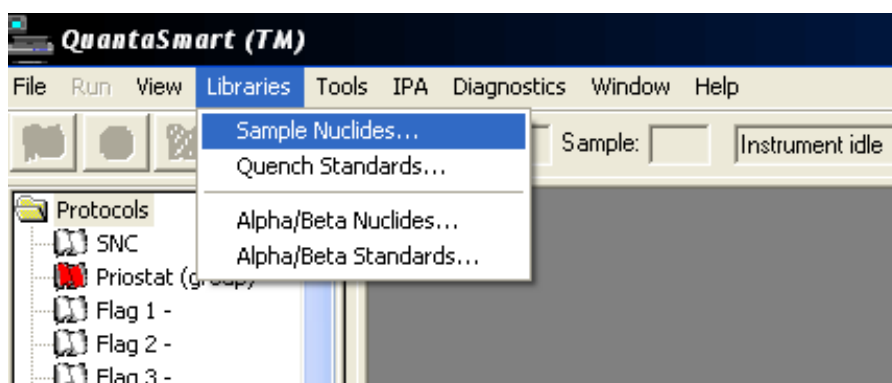
You should set up your sample nuclide in the Sample Nuclide Library before making an assay. (If you fail to do the above the system will prompt you to do so. It is just a lot easier to do it before making a new assay.)

Looking at the Sample Nuclides

Libraries

Sample Nuclides

The sample nuclide table will be found from the QuantaSmart desktop



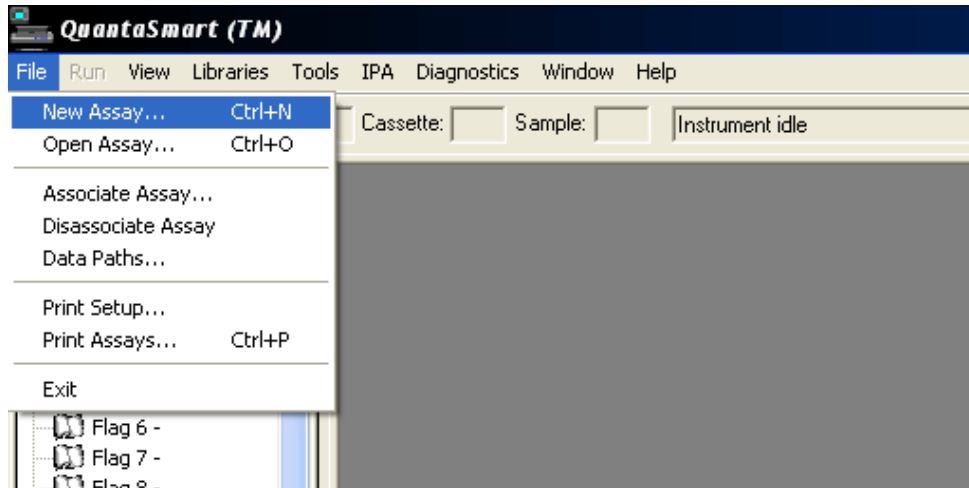
This shows all the information that is associated with the type of sample nuclide with which you wish to work. There can be as many sample nuclides as there is room on the fixed disk. You can choose from factory set ones or you can make your own. A sample nuclide has the regions of interest for the isotope of your choice and also the quench standard set that it will use to calculate the DPM calculation. You can make sample nuclide with a name specific to the analysis that you are doing.

Sample Nuclides								
Nuclide Name	A: LL	A: UL	A: Half-life	A: Units	B: LL	B: UL	B: Half-life	B: U
3H	0.0	18.6	4530.37	Days	2.0	18.6	0.00	Mi
14C	0.0	156.0	5728.49	Years	4.0	156.0	0.00	Mi
3H-14C	0.0	12.0	4530.37	Days	12.0	156.0	5728.45	Y
32P	5.0	1700.0	14.29	Days	50.0	1700.0	0.00	Mi
125I	0.0	70.0	59.24	Days	6.0	70.0	0.00	Mi
3H-125I	0.0	12.0	4530.37	Days	12.0	70.0	59.24	C
3H-32P	0.0	16.0	4530.37	Days	16.0	1700.0	14.29	C

The sample nuclide table allows the user to put in the counting regions A, B and C. Usually a user will only put in region A for general use. If a user requires dual label counting then region A and region B will be used. Rarely will region C be used as it is only for those doing triple label. There are lots of preset sample nuclides and usually one of those will be adequate for most counting situations. If you require a different set of regions to those on the preset list click Add and this will allow you to enter specific regions to your own design.

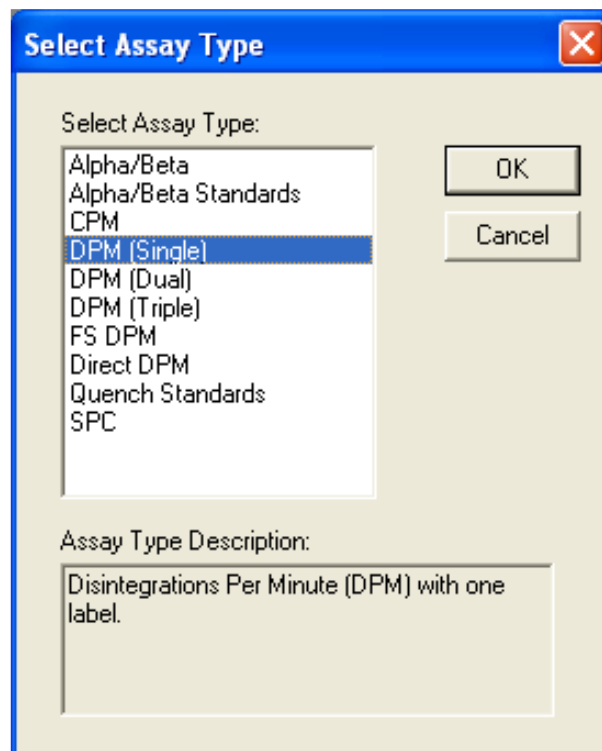
Making a new assay

File
New Assay



Select an assay type.

Here you will chose the type of assay that you will require. For our purposes we will chose DPM (single)



DPM (single) and click OK

You will now go into the Assay Definition page, which is one page, which may have as many as 7 folders on it.

Please be aware that if you enter data and use the Enter key you will immediately engage the save function. Only press the Enter key if you wish to leave the page. Try not to use the Enter key at all.

Assay Definition -

Assay Parameters | Count Conditions | Count Corrections | Report Definition | Report Output | Special Files | Worklist

Assay Type: DPM (Single)

Password: Lock Assay

Author:

Assay Description:

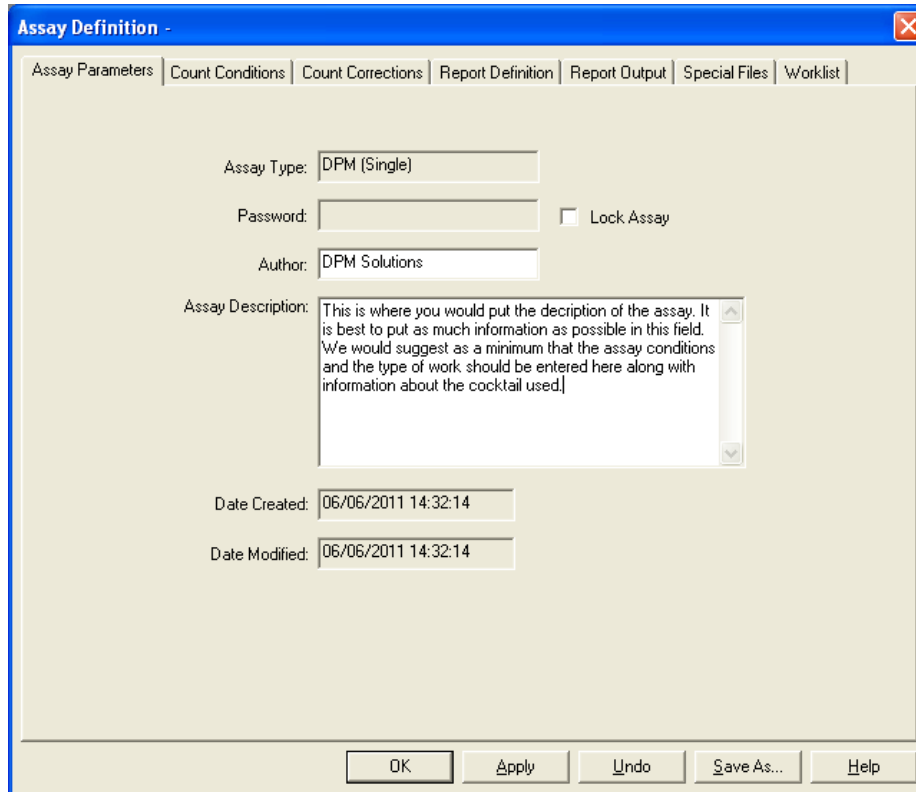
Date Created: 06/06/2011 14:32:14

Date Modified: 06/06/2011 14:32:14

OK Apply Undo Save As... Help

1. Assay Parameters

This allows you to put in an author and a description of the assay.



The screenshot shows the 'Assay Definition' dialog box with the 'Assay Parameters' tab selected. The fields are as follows:

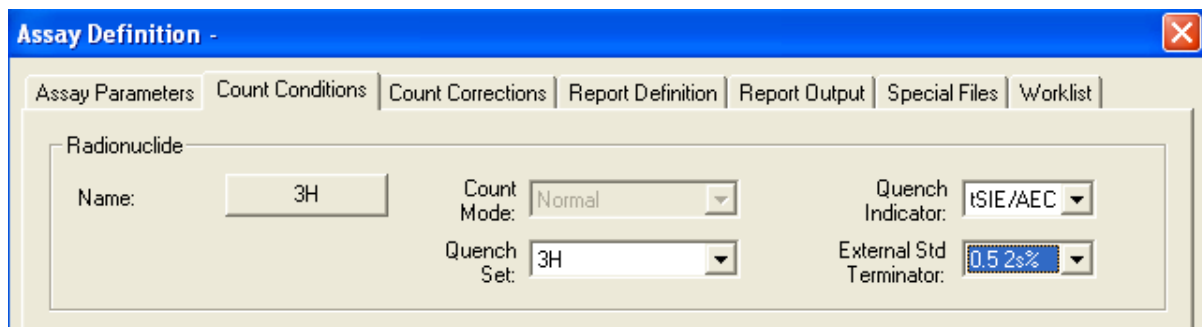
- Assay Type: DPM (Single)
- Password: (empty) Lock Assay
- Author: DPM Solutions
- Assay Description: This is where you would put the description of the assay. It is best to put as much information as possible in this field. We would suggest as a minimum that the assay conditions and the type of work should be entered here along with information about the cocktail used.
- Date Created: 06/06/2011 14:32:14
- Date Modified: 06/06/2011 14:32:14

Buttons at the bottom: OK, Apply, Undo, Save As..., Help.

2. Count Conditions

This is the page that you will use more than any other and most of it is self-explanatory. However, there are some parts that may not be obvious.

At the top there is a section entitled **Radionuclide**.



The screenshot shows the 'Assay Definition' dialog box with the 'Count Conditions' tab selected. The 'Radionuclide' section contains the following fields:

- Name: 3H
- Count Mode: Normal
- Quench Indicator: tSIE/AEC
- Quench Set: 3H
- External Std Terminator: 0.5 2s%

The large button, which has a default of 3H, is the sample nuclide that you have previously set up. Click on the button and you will go to the Sample Nuclide table where you can select the sample nuclide you want. Unless you are an advanced user you may leave the rest of the section as the default settings.

The bottom part of the page there is a section **Count Parameters**.

Regions		
Lower Limit	Upper Limit	
A	0.0	18.6
B	2.0	18.6
C	0.0	0.0

These are the sections that you will be used to changing and involve the time of counting and how many replicates you have. All Packard Tri-Carb software has the same information. It is just displayed a different way.

Pre-count Delay: – beware of using this feature as it will put a count delay on every sample that is in the assay. If you need to dark adapt your samples then put them in the counter and leave them for the desired adaption time before starting the count

Count Time: This is the time period for the counting of your samples

Assay Count Cycles: if you wish your entire assay to count more than once then put the relevant number of times in this box.

Repeat Sample Count: Enter the number of times you would like each sample counted while in the detector. This differs from Assay Count Cycles, where samples are unloaded from the detector and make a complete cycle around the sample changer deck prior to recounting.

Calculate % Reference: Activate the percent reference calculation by marking this box. The instrument reports the value of each sample as a percentage of a reference vial. The reference vial should be the first non-background vial loaded in the cassettes.

Number of Vials per Sample: Enter the number of replicates of each sample being counted. The data output will report the average value of the replicates.

The screenshot shows a software configuration window with four main sections:

- Regions:** A table with columns 'Lower Limit' and 'Upper Limit' and rows 'A', 'B', and 'C'.

	Lower Limit	Upper Limit
A	0.0	18.6
B	2.0	18.6
C	0.0	0.0
- Background Subtract:** A checked checkbox. Below it is a dropdown menu with 'Manual' selected. Underneath are input fields for regions A (0.00), B (0.00), and C (0.00).
- Low CPM Threshold:** An unchecked checkbox. Below it are input fields for regions A (0), B (0), and C (0).
- 2 Sigma % Terminator:** An unchecked checkbox. Below it are radio buttons for 'Any Region' (selected) and 'All Regions'. Underneath are input fields for regions A (0.00), B (0.00), and C (0.00).

Background Subtract: Here you are offered 3 options.

- 1st vial subtract – where the CPM of the first vial is subtracted from all subsequent vials before any DPM calculation takes place
- IPA – here the background recorded when the Tri-Carb performs the SNC operation is used as the background value to be subtracted
- Manual – here you may enter a manual background that you have previously calculated

Low CPM Threshold: If you wish to terminate a count that has a low value (such as the background fractions of a chromatography experiment) then entering a value here will terminate the count if the value is lower than the set value.

2 Sigma % Terminator: Mark this box to activate count termination from statistical accuracy. Two options are available:

Any region - Enter the level of statistical accuracy for each region (as a percent value) that you would like to achieve before counting terminates. Counting terminates when the sigma value of any one region is reached. Using this feature, counting may terminate before the specified count time elapses.

All regions - Enter the level of statistical accuracy for each region (as a percent value) that you would like to achieve before counting terminates. Counting terminates when the sigma value for each region is reached. Using this feature, counting may terminate before the specified count time elapses.

3. Count Corrections

This page allows you to correct for static, luminescence, colour in the sample and heterogeneity in the sample. The half life correction is hardly ever used and please do not touch the coincidence time and delay before burst unless you know what you are doing. It is recommended that you leave this page as it is unless you know that you will have luminescence in your sample or if your samples contain a lot of colour.

Assay Definition -

Assay Parameters | Count Conditions | **Count Corrections** | Report Definition | Report Output | Special Files | Worklist

Special Conditions

Static Controller Coincidence Time (nsec): 18

Luminescence Correction Delay Before Burst (nsec): 75

Colored Samples

Heterogeneity Monitor

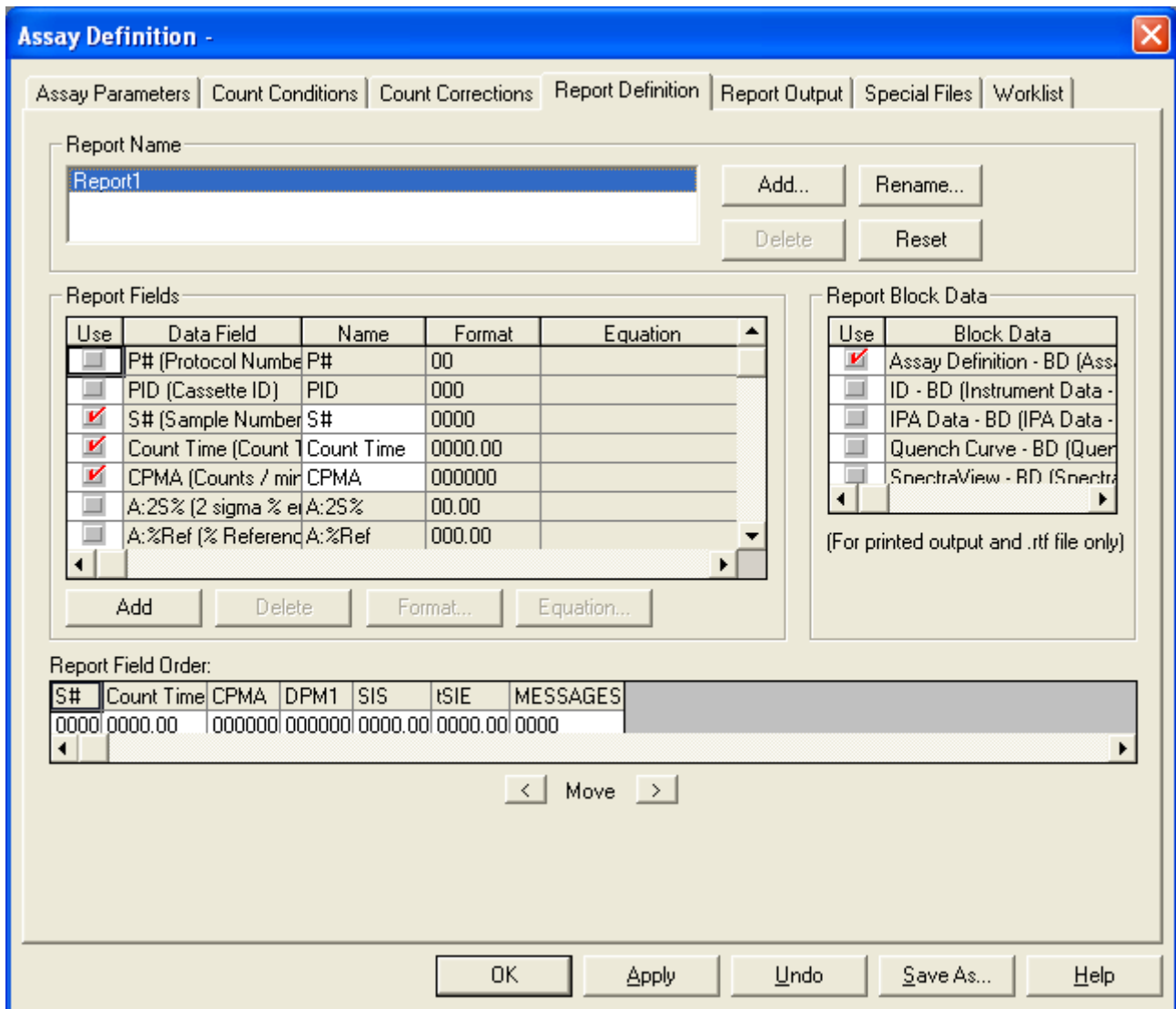
Apply Half-life Correction

Nuclide: 3H

	Lower Limit	Upper Limit	Half-life	Units	Reference Date	Reference Time
A	0.0	18.6	4530.37	Days	Start of Assay	Start of Assay
B	2.0	18.6	0.00	Minutes	Start of Assay	Start of Assay
C	0.0	0.0	0.00	Minutes	Start of Assay	Start of Assay

OK Apply Undo Save As... Help

4. Report Definition.



You are able to have more than one report format and can print out as many different reports as you like. You can also name the reports in any way you wish. Along the bottom of the page is the Report Field Order. This shows you how the report will be printed out. To add to the report print out all you have to do is tick the box in the Use on the Report Fields. This will change to red and then it will be displayed on the Report Field Order. To move it to the position that you require highlight it and then use the Move keys on the bottom to position it. This page is not as difficult as it looks at first glance.

Usually the default set up is the one that is best for you.

5. Report Output.

You are able to output the data either as a data file, an RS232 file, or a Rich Text File. For a data file you can export it in ASCII format or Excel format. You can simultaneously export it as a Rich Text File, which can be read by any word processing software. On this example the file will be exported as a Delimited Text which may be easily exported to Excel.

Assay Definition -

Assay Parameters | Count Conditions | Count Corrections | Report Definition | **Report Output** | Special Files | Worklist

Report Name: Report1

Output To Printer

Data File

File Type: Delimited Text

File Name: Report1.txt

Output Per: Page

Include Column Header

Rich Text File

File Name: Report1.rtf

Run Application

Application: []

Data File: None

Run After Each: Batch
 Cycle
 Sample

RS-232

Output Per: Sample Average

Additional Data:

Protocol Data
 Spectrum Data
 Column Header

Alternate Data Formats:

Tri-Carb 2100/2300 Format
 Tri-Carb 2500/2700 Format

OK Apply Undo Save As... Help

6. Special Files

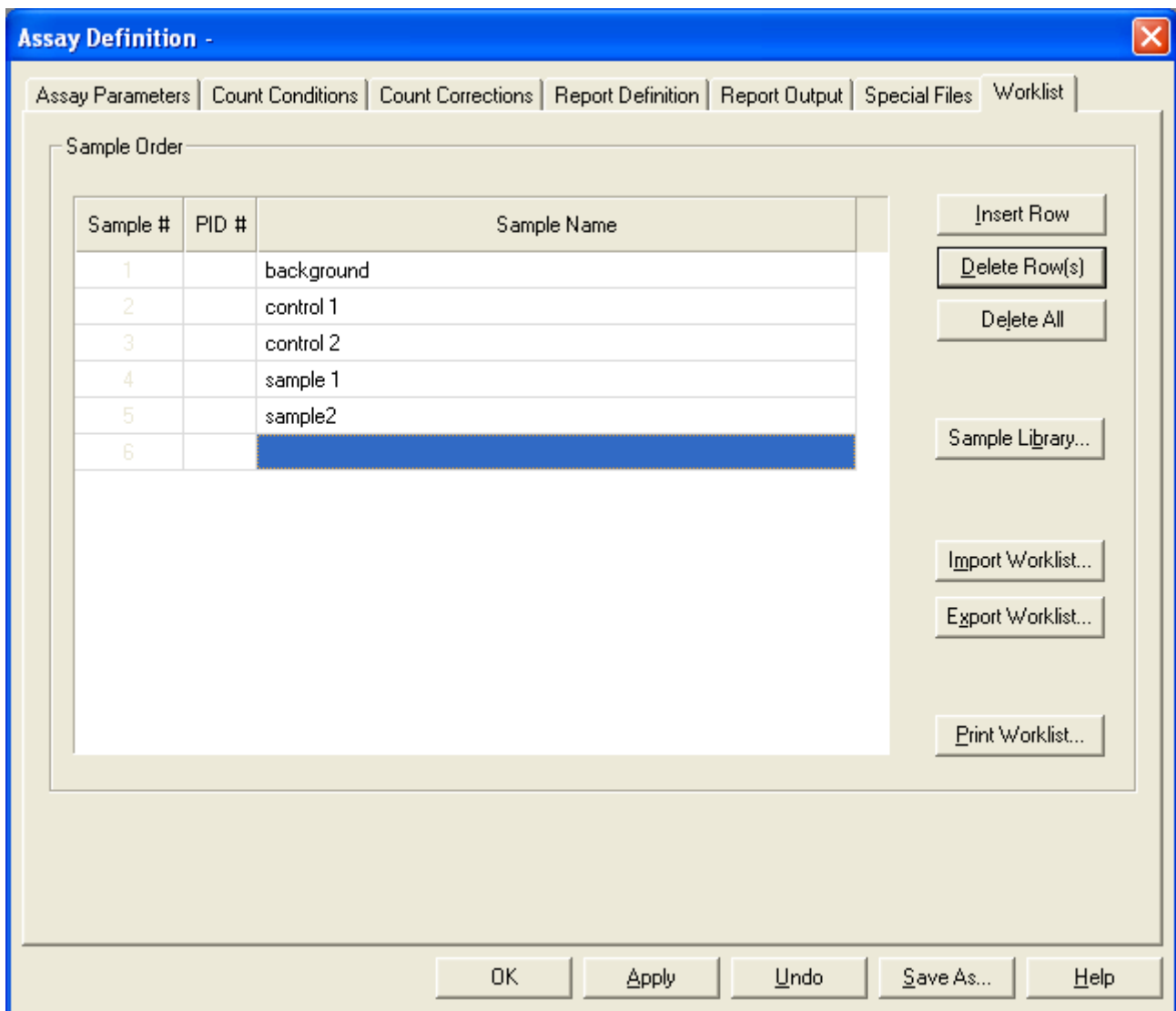
This page is rarely used. It allows you to save Spectra. It may be useful if you want to display the Alpha/Beta spectra when doing gross Alpha- Beta separation. Otherwise the page is not often used.

The screenshot shows the 'Assay Definition' dialog box with the 'Special Files' tab selected. The dialog has a blue title bar and a standard Windows-style border. The 'Special Files' tab is highlighted in the tab bar at the top. The main area contains several sections, each with a checkbox and a text input field:

- Spectra File (composite)
File Name:
- Spectrum Files (individual)
File Name: P = flag number
S = sample number
.### = repeat count number
- IPA Data File (Current Values)
File Name:
- Prot.dat File
- 2000CA.dat File

At the bottom of the dialog, there are five buttons: OK, Apply, Undo, Save As..., and Help.

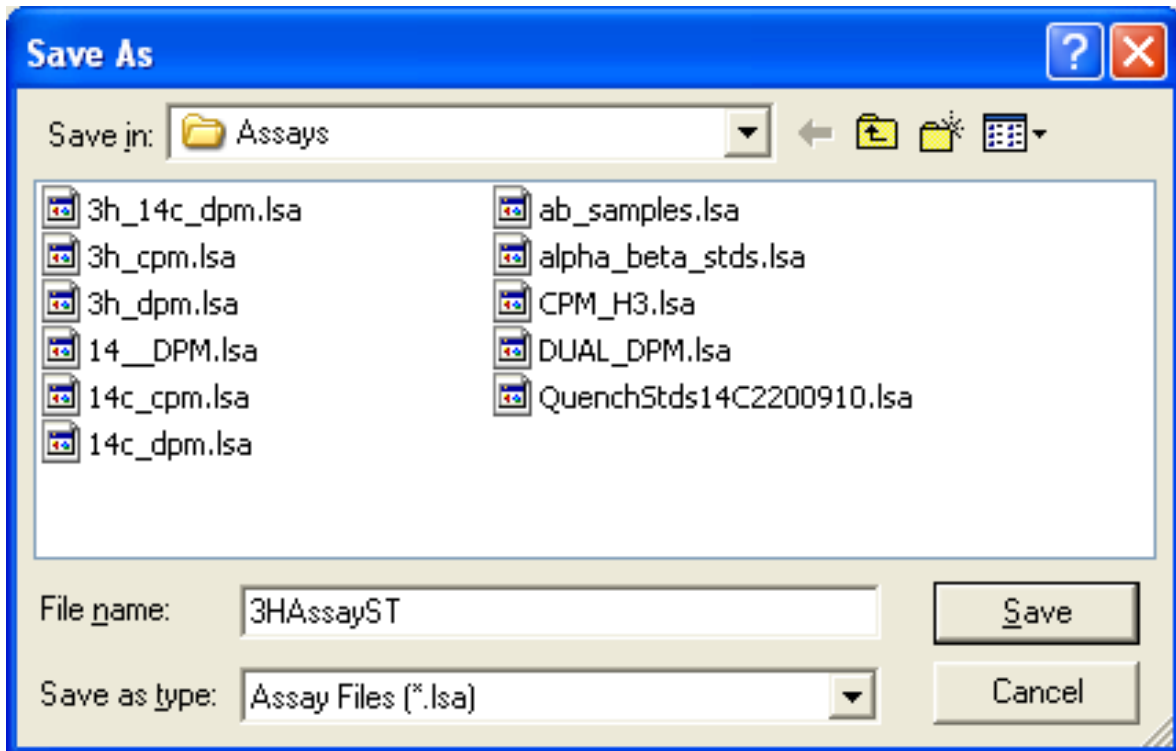
7. Worklist (if the option has been purchased)



This allows the print out (either on the printer or in the data output) to have a unique name with each sample. You are able to give each sample you count a name or number. It has the limitation that if you wish to edit a worklist you may only do so from within the assay. It will not import worklists from programmes such as Excel. A sample library may be built from within QuantaSmart to have a range of ready-made names for you to use that are common to your work.

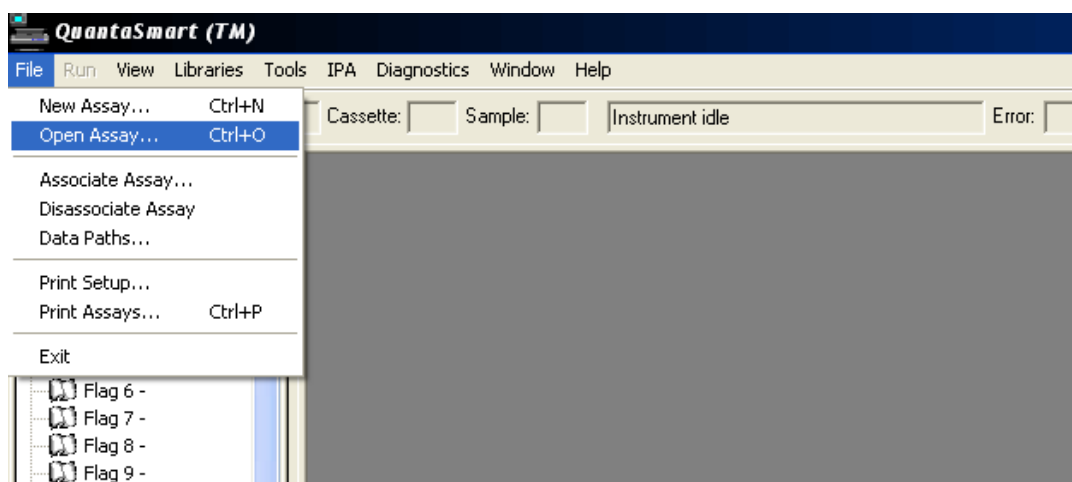
Saving the assay

When you have entered all the parameters in the assay you will click on the OK button. This will take you to a Save As function. Type in your File name and your assay will be saved. Here the assay will be saved under the name 3HAssayST.

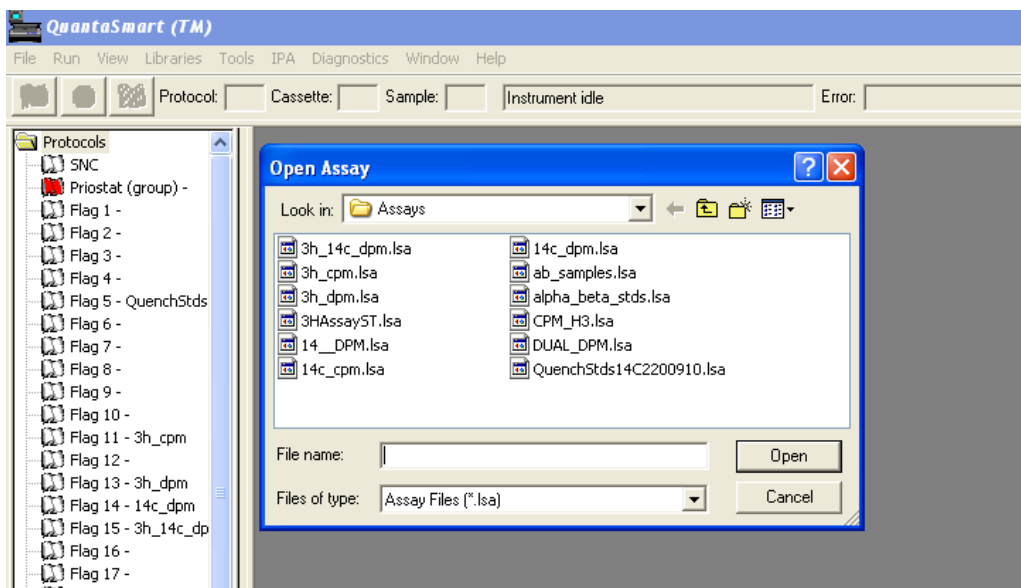


Open an existing assay

File
Open Assay



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This allows you to open an existing assay and then allows you to work as in the details above. At the end you can save it to the existing file name or you can change the file name when you leave the file. You only need to change the part you need and there is no necessity to work through the whole file to get to the part you want. Just go straight to it, change it and then resave the file.

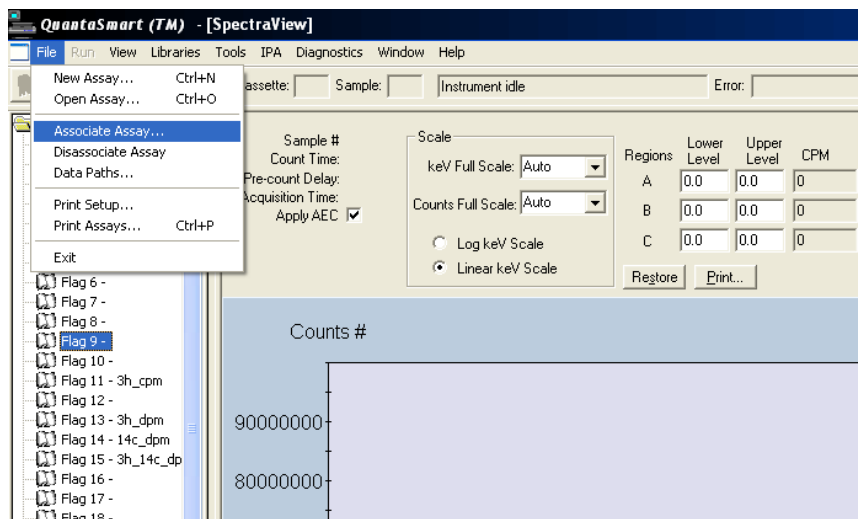
Running the Assay

Getting ready to run the assay

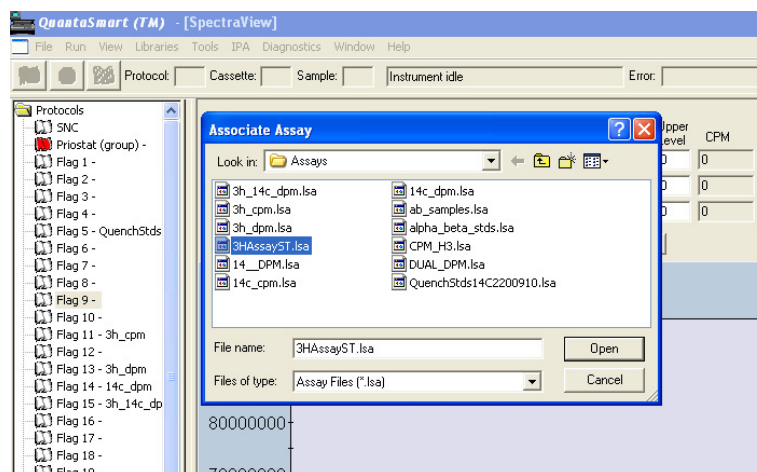
When you have made your assay you need to associate it with a flag (the same flag we used to call the “protocol flag”) and then the LSC acts as it did before.

Associating an assay

First highlight a flag position of a vacant flag down the left hand side of the protocols (main) screen. When the flag is highlighted then move to the steps below.

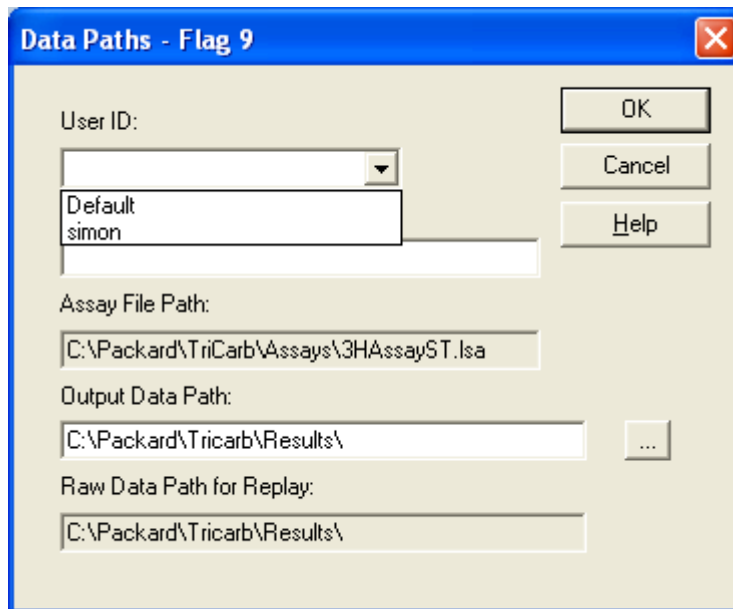


File
Associate assay



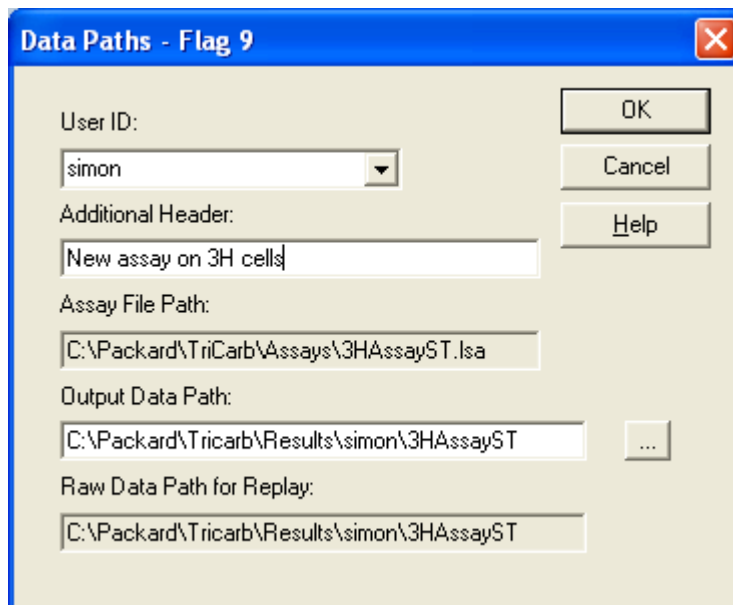
You will see all the available assays. Chose the assay you want to run. In this case 3HAssayST has been selected.

You will then be prompted for the User ID. If this is the first time you have used the LSC then type in your name or ID number. If you have used it before then use the dropdown arrow to give you a list from which to choose a name. In this case we will choose Simon.



The screenshot shows the 'Data Paths - Flag 9' dialog box. The 'User ID' field has a dropdown menu open, displaying 'Default' and 'simon'. The 'Assay File Path' is 'C:\Packard\TriCarb\Assays\3HAssayST.lsa'. The 'Output Data Path' is 'C:\Packard\Tricarb\Results\' with a browse button. The 'Raw Data Path for Replay' is 'C:\Packard\Tricarb\Results\'.

The Additional Header is information you can add on this occasion only which is printed on the report.

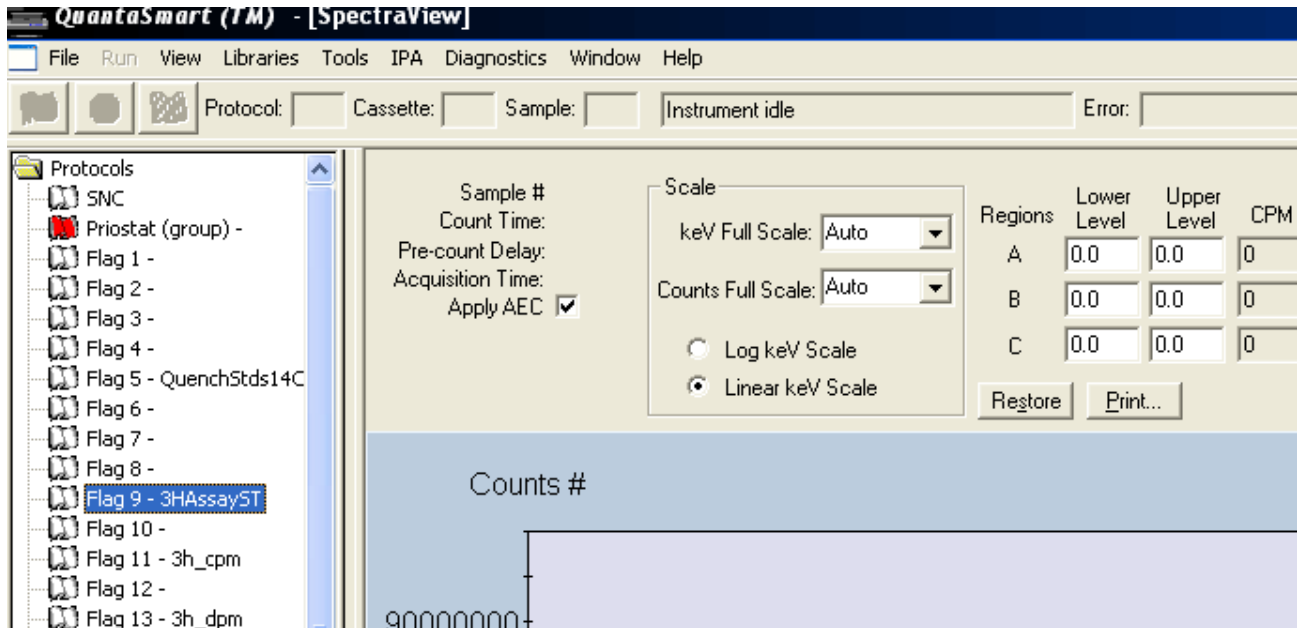


The screenshot shows the 'Data Paths - Flag 9' dialog box. The 'User ID' dropdown menu is set to 'simon'. The 'Additional Header' field contains 'New assay on 3H cells'. The 'Assay File Path' is 'C:\Packard\TriCarb\Assays\3HAssayST.lsa'. The 'Output Data Path' is 'C:\Packard\Tricarb\Results\simon\3HAssayST' with a browse button. The 'Raw Data Path for Replay' is 'C:\Packard\Tricarb\Results\simon\3HAssayST'.

Click OK

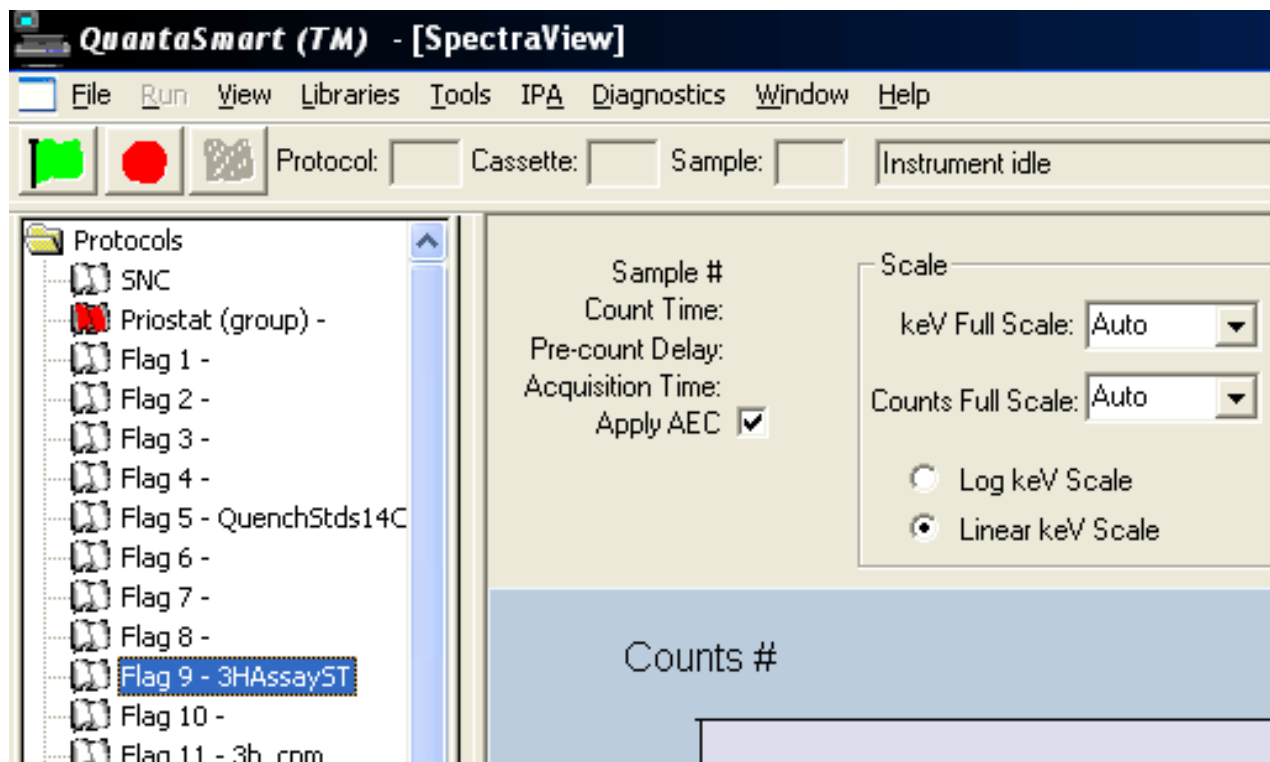
The Flag you chose will now be highlighted with the assay you chose.

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Put the correct flag in the cassette and load your samples with the first one at the flag end of the cassette. Load the cassette in the LSC on the right hand side of the sample deck

Press the Green flag at the top. The LSC will search for the flag and the assay will be run.



Disassociating an assay

When the run has finished and you have recovered your samples you may disassociate the assay from the flag. To do this, follow the instructions below.

Highlight the flag you wish to disassociate.

File

Disassociate assay

The flag will now become clear and ready for someone else to use.

Stopping an assay

If you wish to stop an assay half way through the run then use the Red stop button at the top. This action will only stop the assay – it will not end it and it will leave the sample and cassette in the place you stopped the assay.

Ending an assay

When the assay has counted the cassette will process round the deck of the counter until either another cassette with a different protocol is identified by the counter or the assay you have just counted goes all the way round and is seen by the sensors again. If the assay has only requested one cycle then the counter will know the assay is completed and the counter will print out the result and or send the data for storage. If you want to end an assay without waiting for it to process round the deck you can use the chequered flag button and end the assay. This is useful if you have seen enough and want to run under different conditions – like a longer run time.

Using QuantaSmart without associating or disassociating the flag

If you do not wish to alter the settings for any assay you can leave it permanently associated with an assay and flag. The assay will continue to run as if it was the 2000 series of Tri-Carbs.