TECH TIP # 70

TMT data acquisition on the LTQ-Orbitrap XL Mass Spectrometer

TR0070.0

Introduction

Successful acquisition of quantitative tandem mass tag (TMT*) data with the Thermo Scientific LTQ-Orbitrap XL Mass Spectrometer requires several considerations. As many peptides as possible should be analyzed, and reporter ions (found in the low mass region of MS/MS spectra) must be acquired with sufficient intensities for good quantitative analysis. Careful adjustment of instrument settings and acquisition parameters must be made to obtain these data. These settings are detailed in this document.

Preparing for the experiment

A. Materials Required

- Binding buffer (0.1 to 1.0% TFA in water)
- Formic Acid for the mobile phase
- Bottled HPLC water
- Acetonitrile
- Capillary LC column (75µm × 20cm C18 column is recommended)
- Liquid Chromatograph Instrument capable of flow rates down to 250nL/min.

B. Procedure

1. Prepare fresh mobile phases.
2. Use a fresh column that has been well equilibrated. The column length should be at least 20cm.
3. Calibrate the mass spectrometer. It is especially important to calibrate the positive ion electron multipliers. Doing so will ensure that the instrument is operating with maximal sensitivity.
4. Dilute all samples with binding buffer. Target loading amounts to be in the 0.25 to 1.0µg range.

Preparing the LC method

Proper setup of the LC method is critical to the success of the experiment. While LC methods vary widely, several common points have emerged:

1. Sample complexity determines the choice of gradient. Simple samples (e.g., from immunoprecipitation or enrichment experiments) require short 1-hour gradients (usually 4 to 40% B (acetonitrile/0.1% formic acid) while more complex samples require much longer gradients (up to 3 hours; 4 to 40% B over 3 hours).
2. Flow rates should be set to 250 to 600nL per minute.
3. Trap columns should be avoided as they tend to lose sample.
4. Always run a labeled positive control sample before analysis of the test sample. Be sure that the chromatographic peaks are less than 30 seconds wide (on average). If the peak widths are broad, change the LC gradient until your peaks sharpen.
5. Also, the more pre-fractionation done before hand (e.g., SCX fractionation), the more likely the experiment will be successful. This will help to ensure the under-sampling is minimized.

Preparing a suitable Tune File

Proper setup of the tune file is essential for a successful experiment. All tune file parameters are entered in the Tune window.

1. First adjust the Scan Time Settings (Setup → Injection Control).

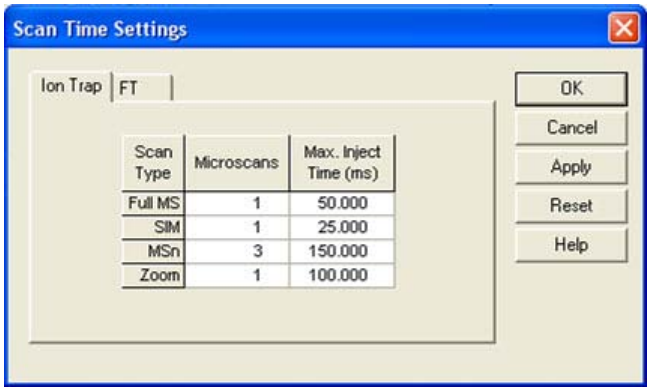

Figure 1. Scan Time Settings for the Ion Trap. While the quantitation is done in the HCD cell and mass measurements are done in the Orbitrap, MS/MS spectra (CID) will be measured in the Ion-Trap.

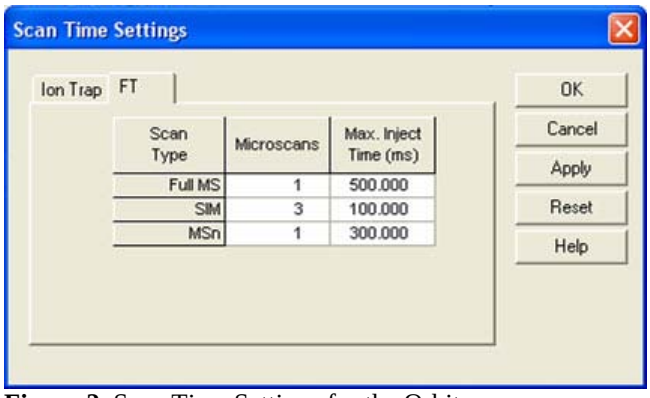

Figure 2. Scan Time Settings for the Orbitrap.

2. Next, enter the appropriate target values for both the Ion trap and the Orbitrap (FT) (Figures 3, 4).

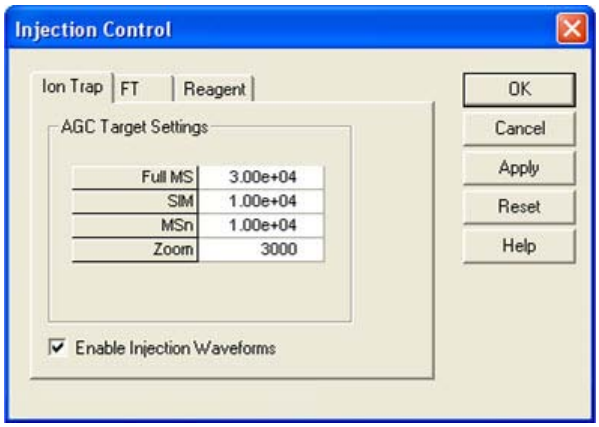

Figure 3.

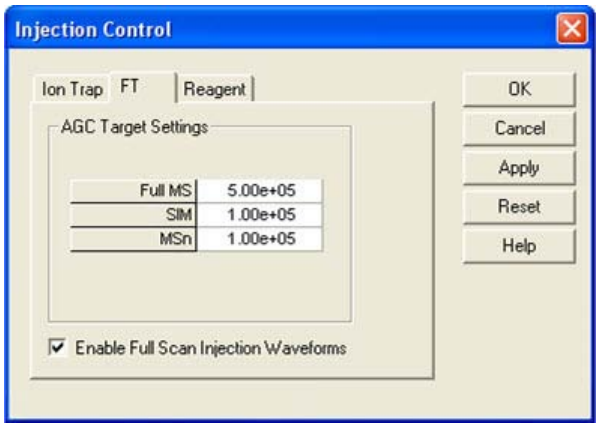

Figure 4.

3. Note that some of the parameters are adjustable, especially the MSn parameters. For example, a scan time of 300ms for the FT-MSn is generally adequate. However, if the sample amount is low (less than 250ng on column), then increasing the scan time by 50 to 100ms is advisable.
4. Save the tune file since you will need to point to this exact file in the acquisition method.
5. Calibration prior to analysis is essential, especially the electron multipliers, in order to obtain maximal sensitivity. Failure to calibrate will lead to large CV's of technical replicates. If you are unfamiliar with the calibration process, consult the instrument documentation. Successful calibration will result in a green check mark next to the parameter in question (Figure 5).

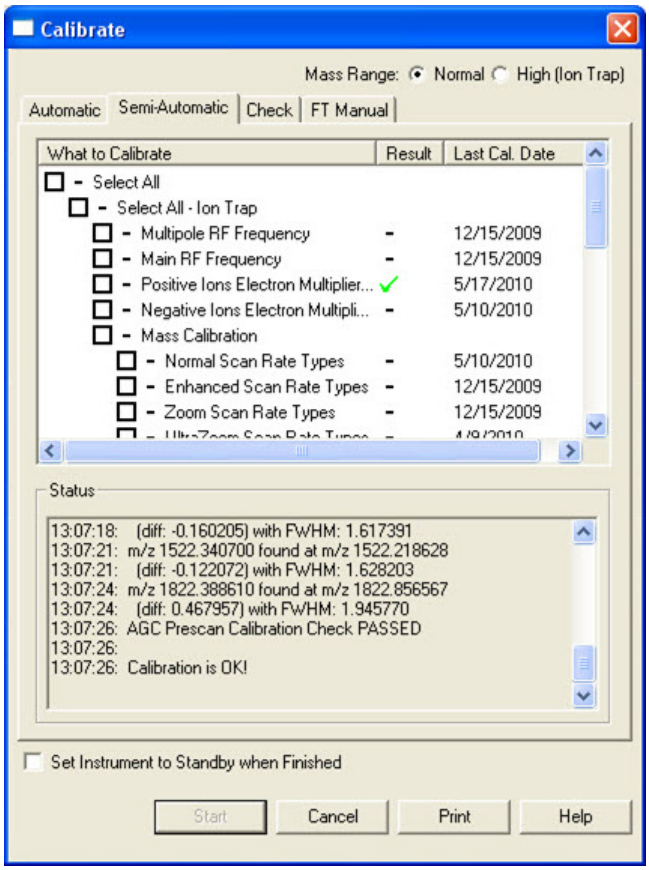

Figure 5.

Constructing an acquisition method

The following method has been developed to maximize protein identification with quantitation. The method is a top 3 × 2, which consists of three HCD events followed by 3 CID events on the same ions selected for the first 3 HCD events. Use the following procedure to systematically set up the method.

1. Go to the Xcalibur software program and click on the Instrument Setup Icon (Figure 6).
2. Right click on the mouse button and select Go to.
3. A new page will appear (Figure 7).

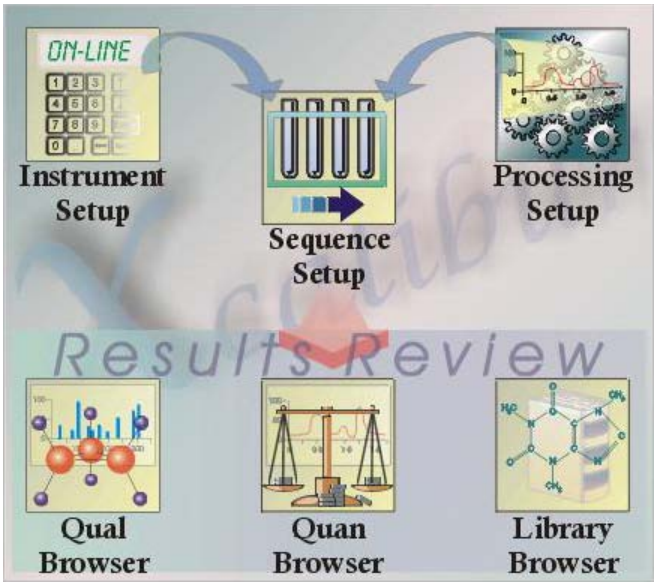

Figure 6.

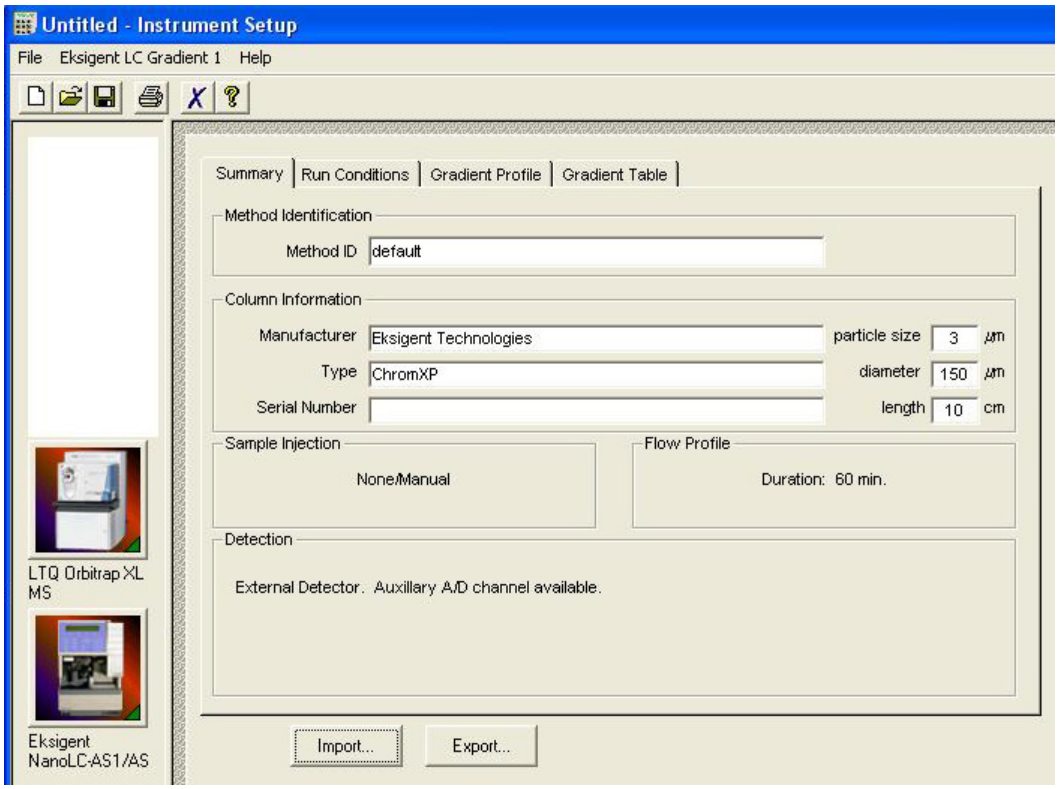

Figure 7.

4. Set up the LC method according to the manufacturer's instructions.
5. Select the MS/MS instrument icon and then click on the icon called Data dependent MS/MS.
6. A new screen will appear. Enter the parameters as they appear in Figure 8 for Scan event 1. Select the Tune method that was saved in the last section and be sure to enter an Acquire Time that is the same length as the LC gradient (can be shorter, but not longer). In this method we are implementing seven scan events. Scan event 1 is the precursor selection step, scan events 2-4 are the HCD steps and 5-7 are the CID steps. In this example, we have set the resolution to 15000. However, higher resolutions in scan event 1 are recommended for highly complex samples so that a maximal number of peptides can be selected.

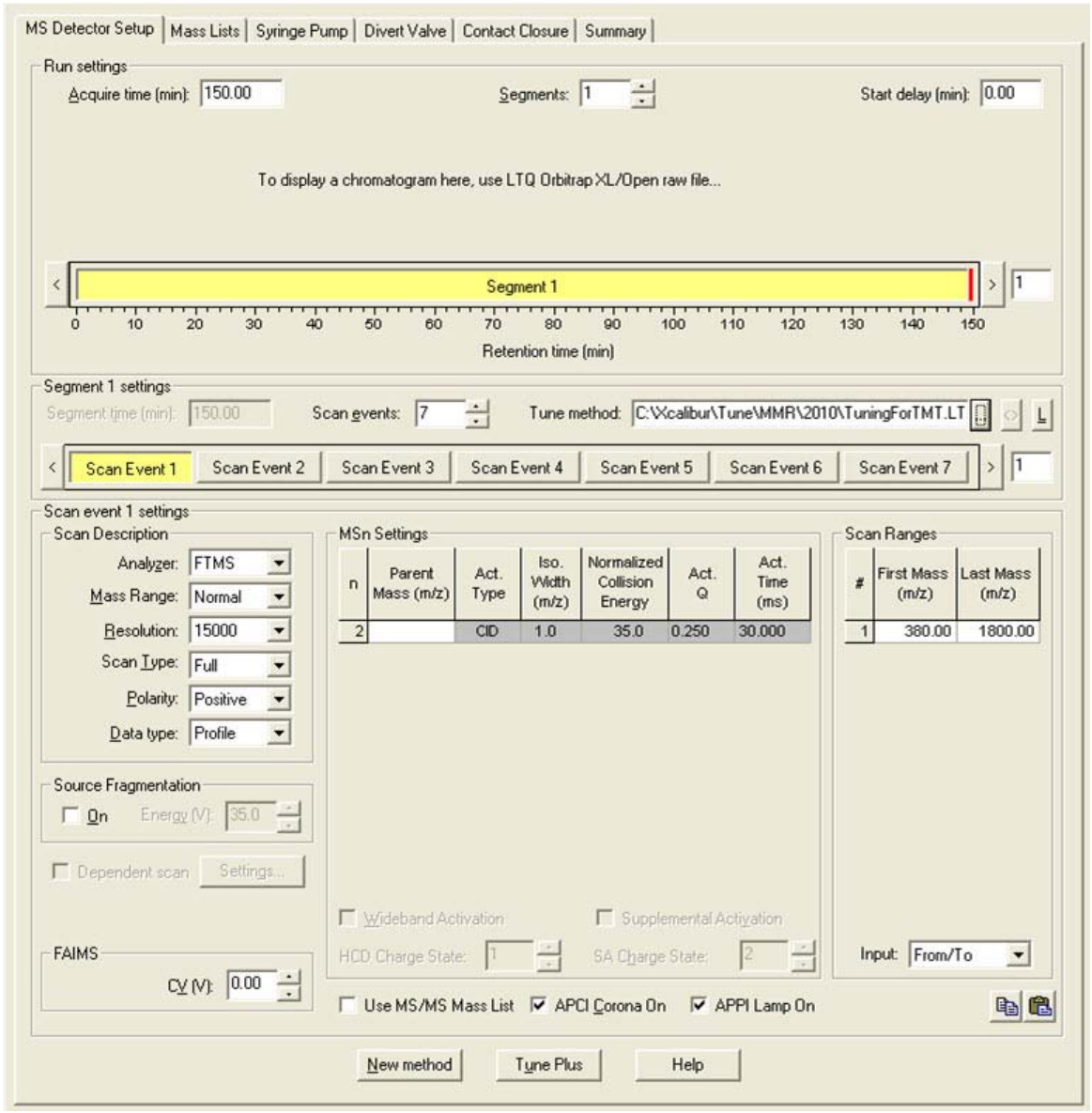

Figure 8.

7. Click on Scan Event 2 and enter the parameters as shown in Figure 9.

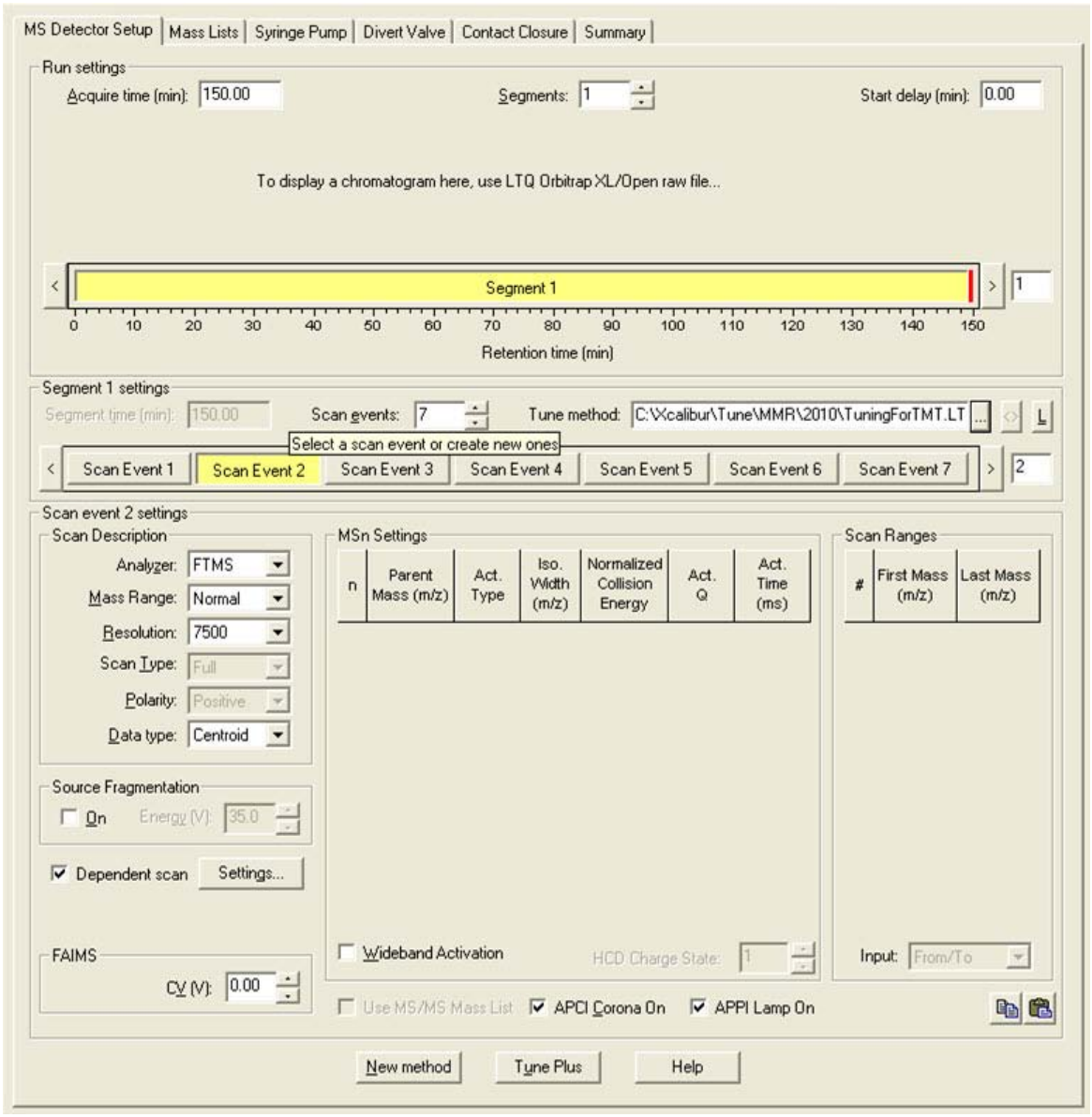

Figure 9.

8. Continue to enter the parameters for the other scan events described here.

Table 1. Settings for Scan Events 3 to 7. All other settings are the same as for Scan Event 2 (Figure 9).

Scan Events 3 & 4 (same as 2)		**Scan Events 5 to 7**	
Analyzer	FMTS	Analyzer	Ion Trap
Mass Range	Normal	Mass Range	Normal
Resolution	7500	Scan Rate	Normal
Scan Type	(off)	Scan Type	(off)
Polarity	(off)	Polarity	(off)
Data Type	Centroid	Data Type	Centroid
Dependent Scan	Checked (on)	Dependent Scan	Checked (on)

9. Click on Scan Event 2. Select the Dependent Scan setting box and then click on the Settings tab. A box will appear (Figure 10).

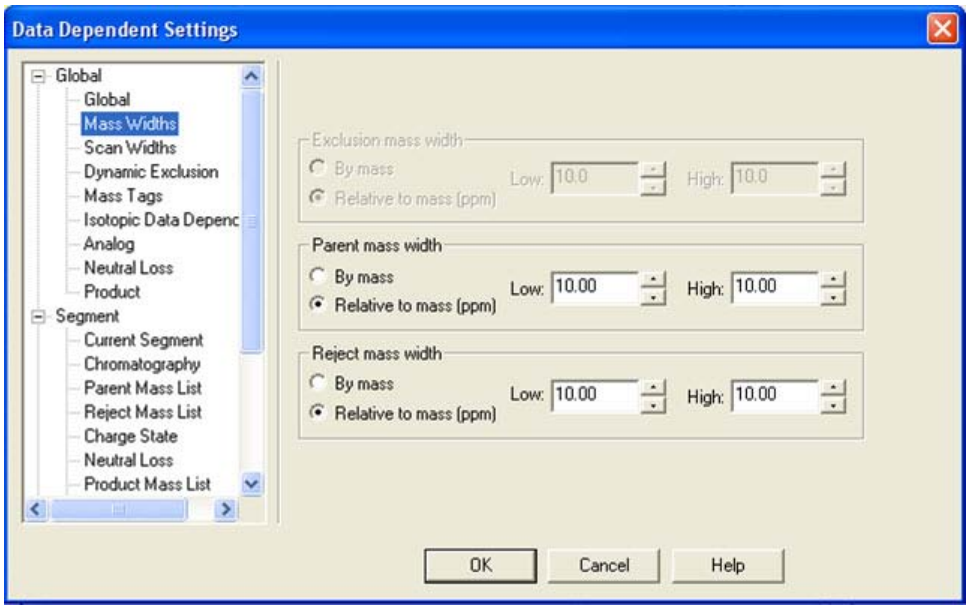

Figure 10.

10. Enter the parameters as shown in Figures 11 to 12.

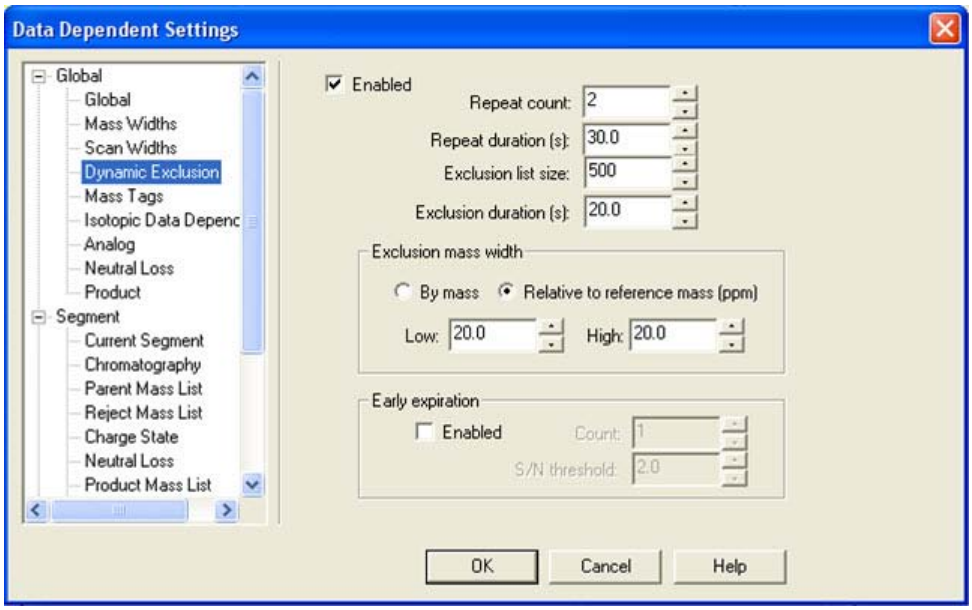

Figure 11.

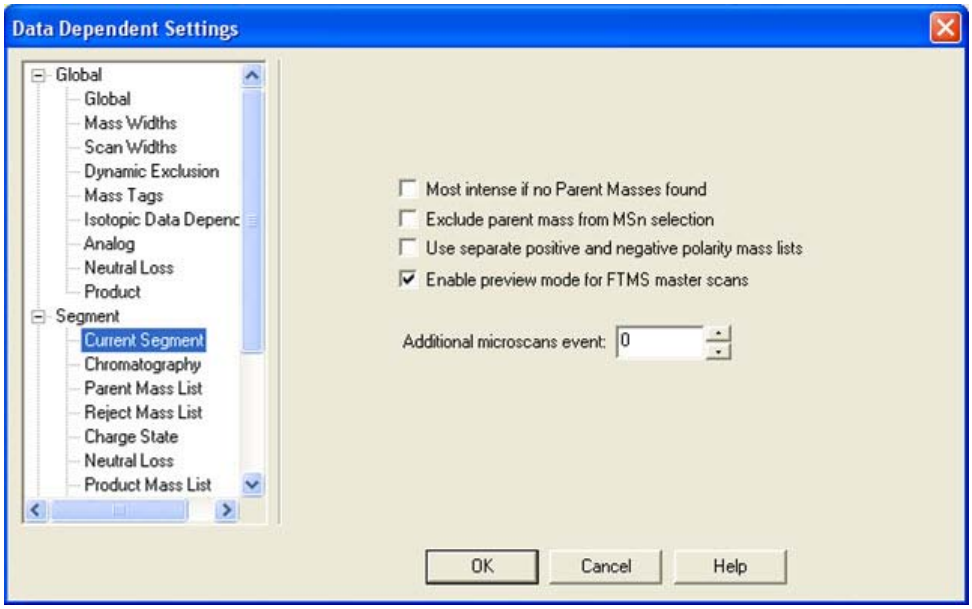

Figure 12.

11. Figure 13 (Reject Mass List), is not required. But, if you have some background ions that you would prefer to ignore, it is recommended to include this step.

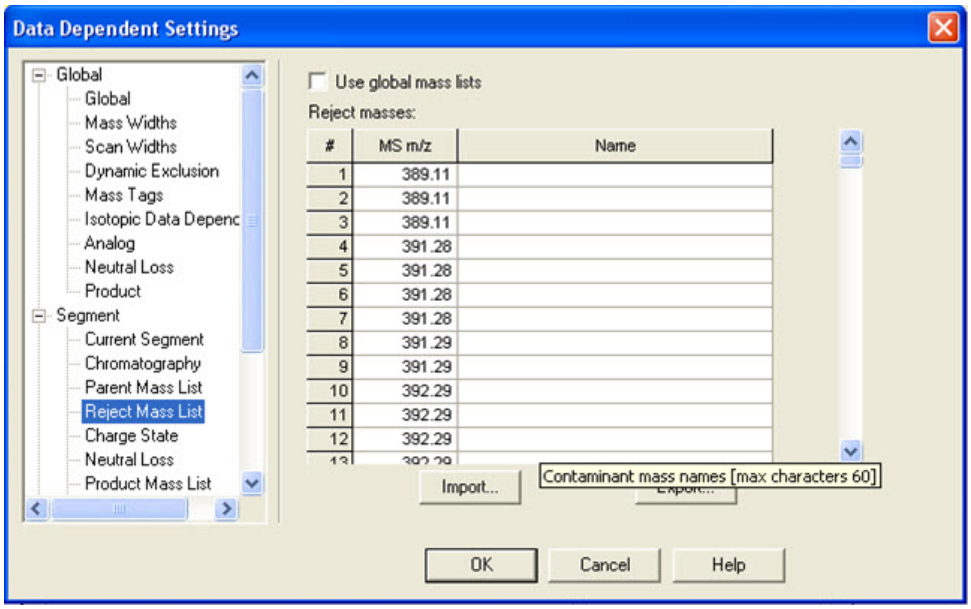

Figure 13.

12. Set up the charge state screening parameter as shown in Figure 14. The use of monoisotopic precursor selection and non-peptide monoisotopic recognition is optional.

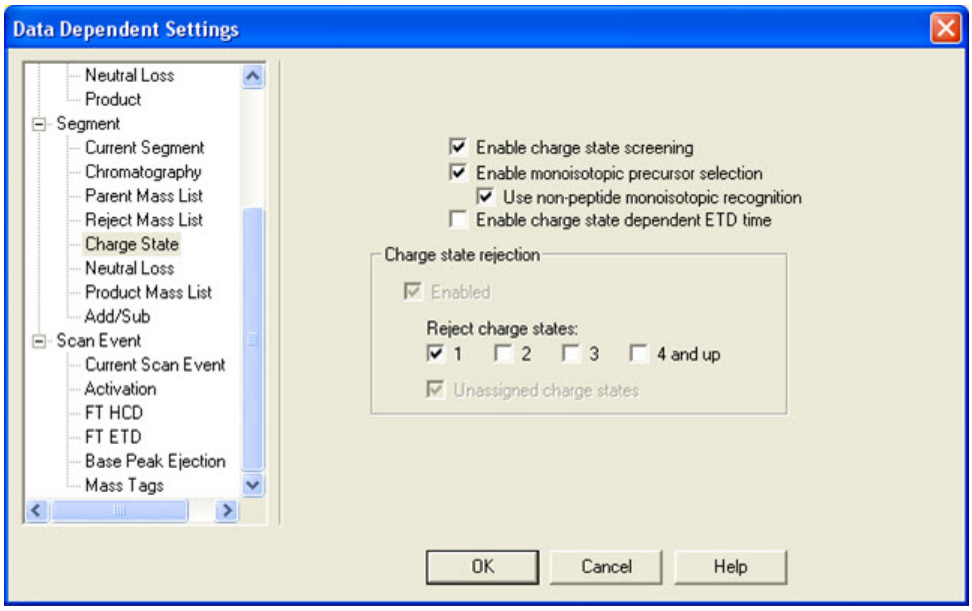

Figure 14.

13. While still in the Data Dependent Settings box, click on the Current Scan Event tab. Enter the values as shown in Figures 15 to 17. These values apply to Scan event 2.

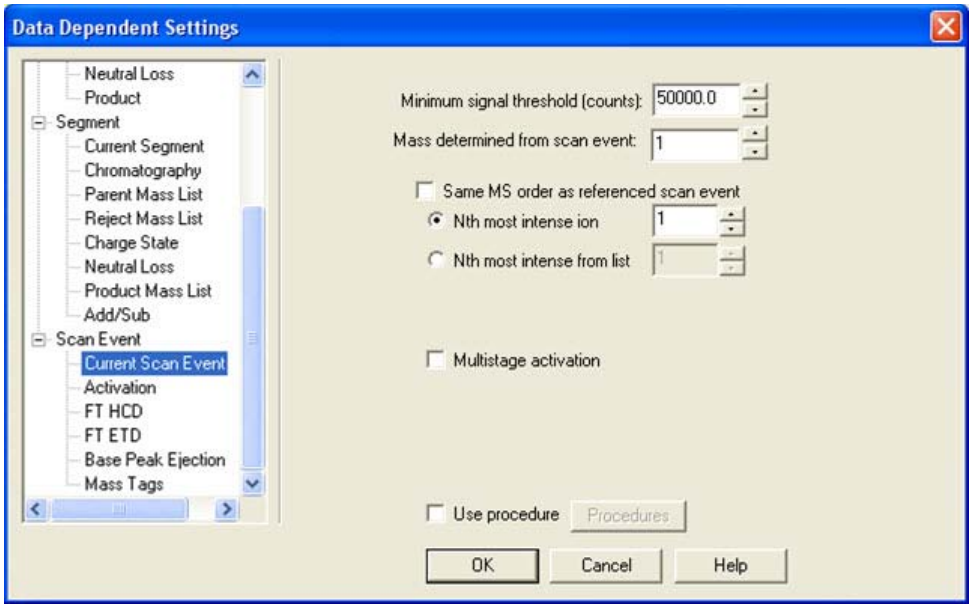

Figure 15.

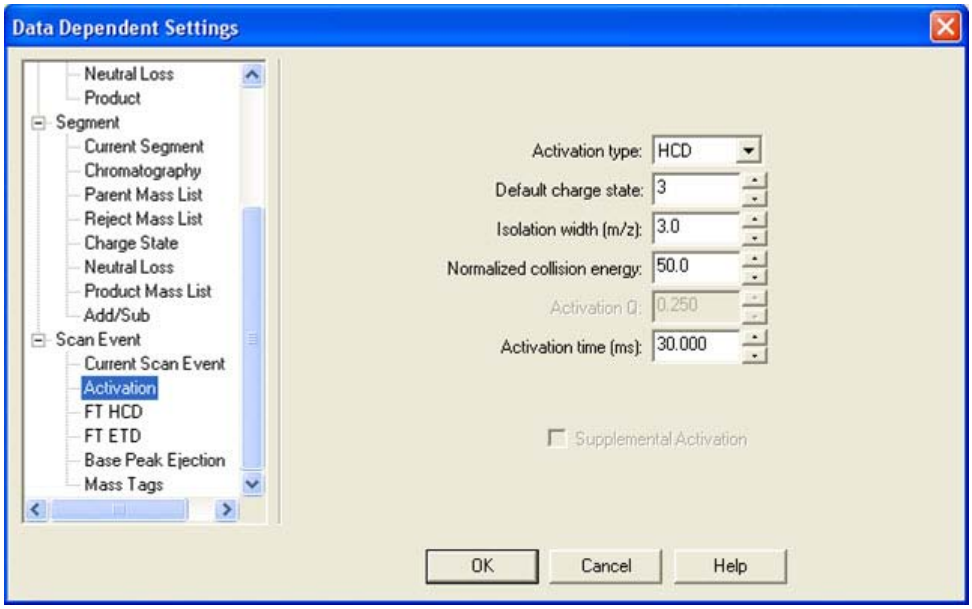

Figure 16.

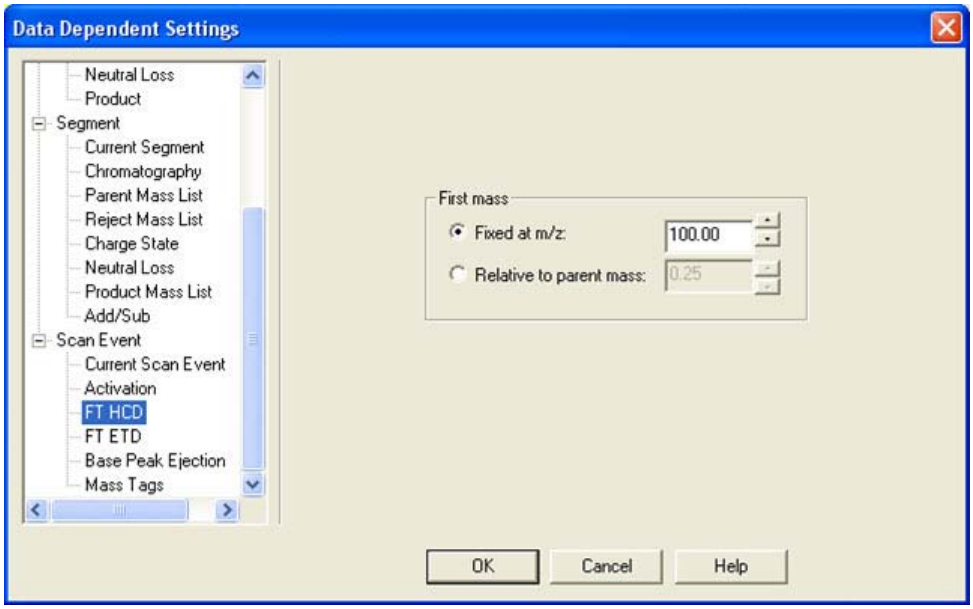

Figure 17.

14. For Scan events 3 and 4, you will want to keep the same Activation and FT HCD values. Apply the values shown in Figure 18 for Scan event 3 and Figure 19 for Scan event 4.

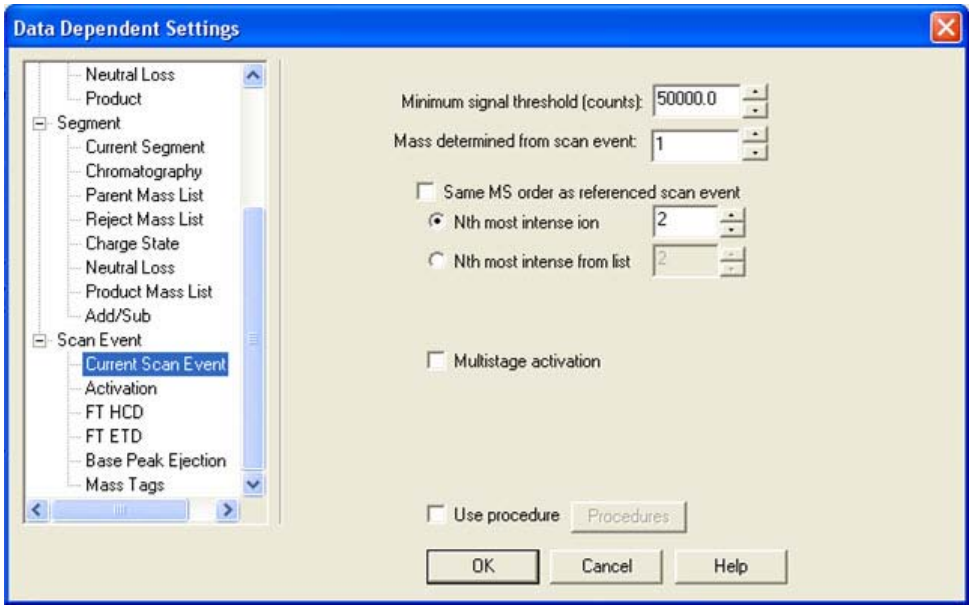

Figure 18.

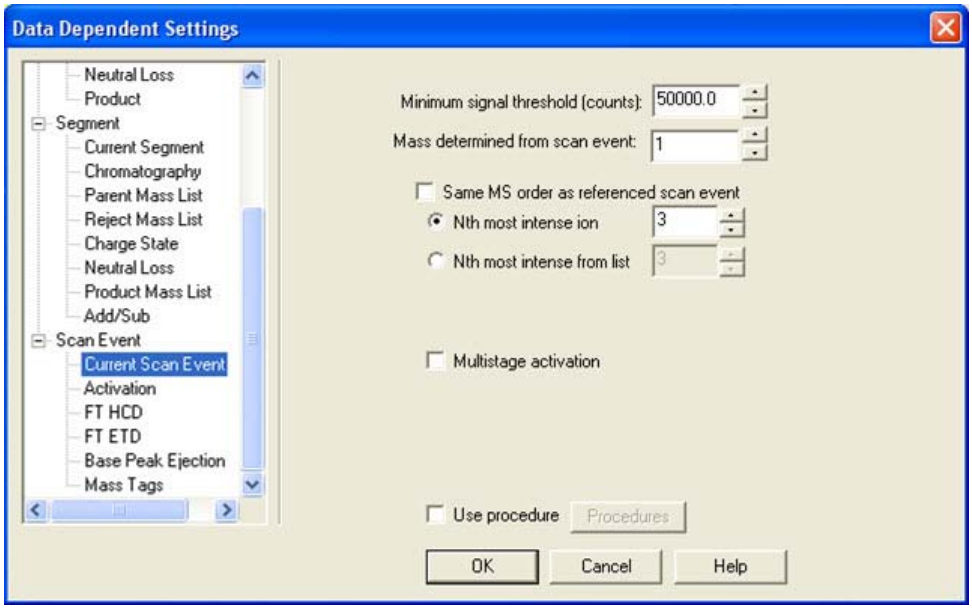

Figure 19.

15. Figures 20 to 22 apply to Scan Events 5, 6 and 7. These are the CID steps in the duty cycle.

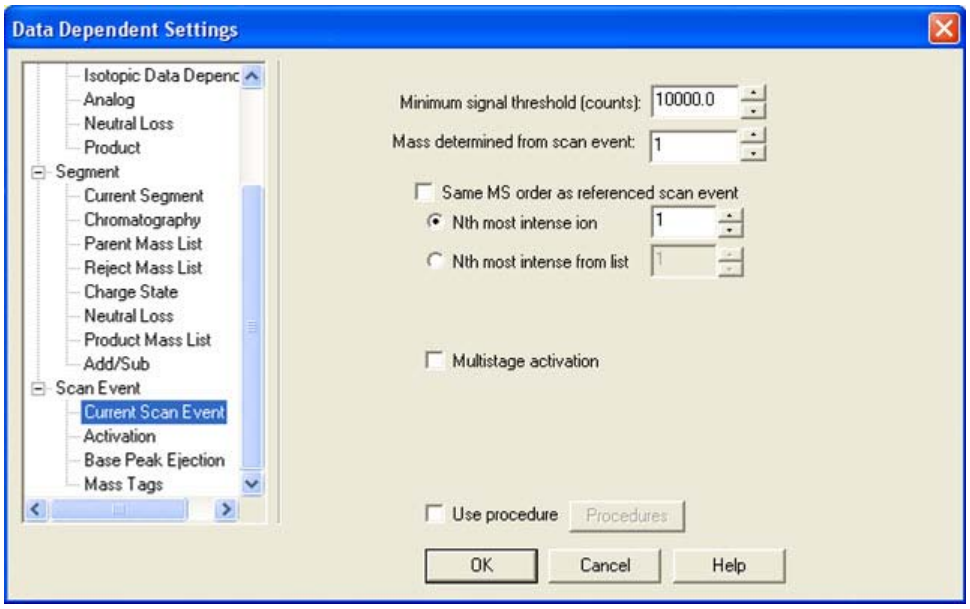

Figure 20.

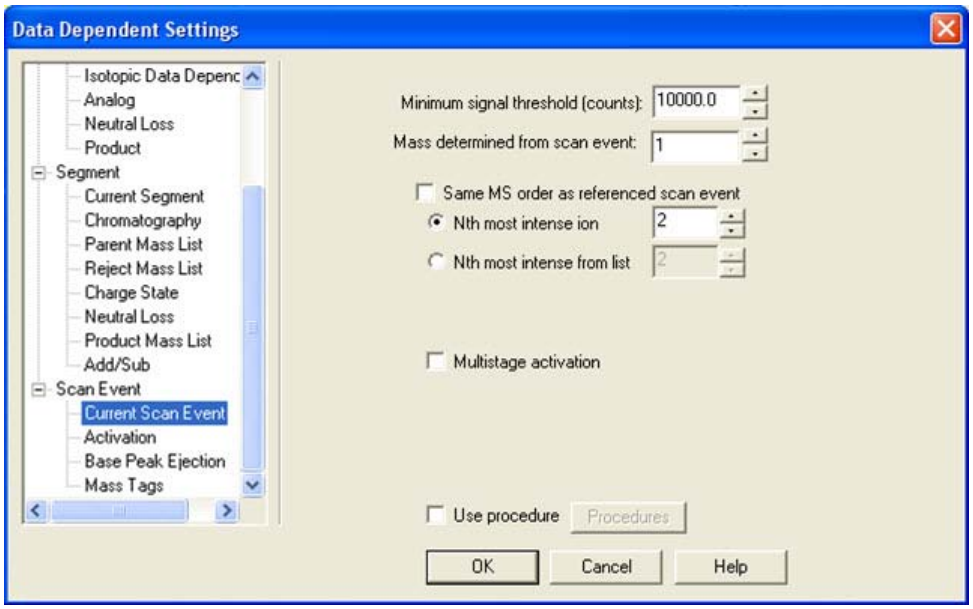

Figure 21.

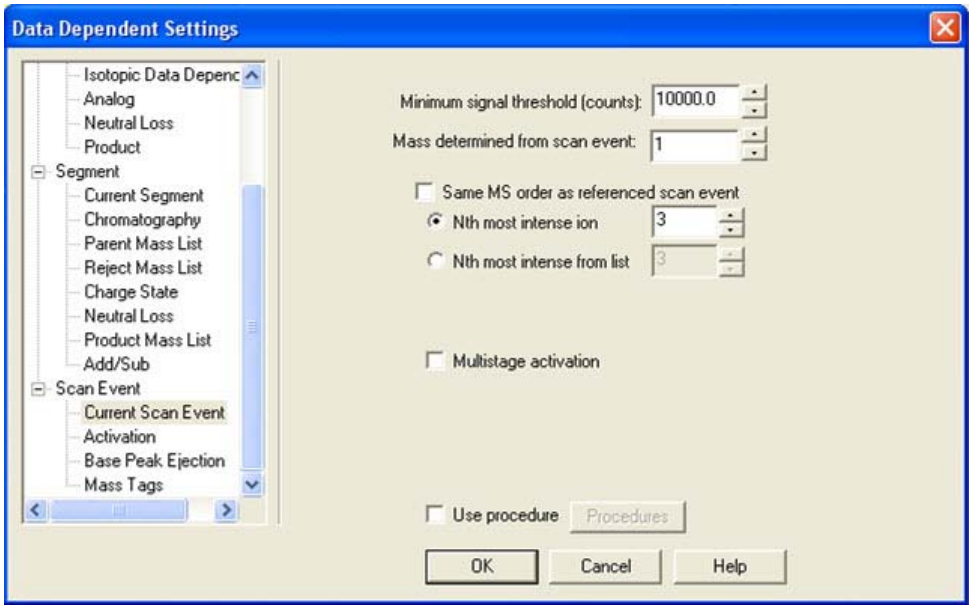

Figure 22.

16. For each of the scan events 5 to 7, the activation tab will require the values shown in Figure 23.

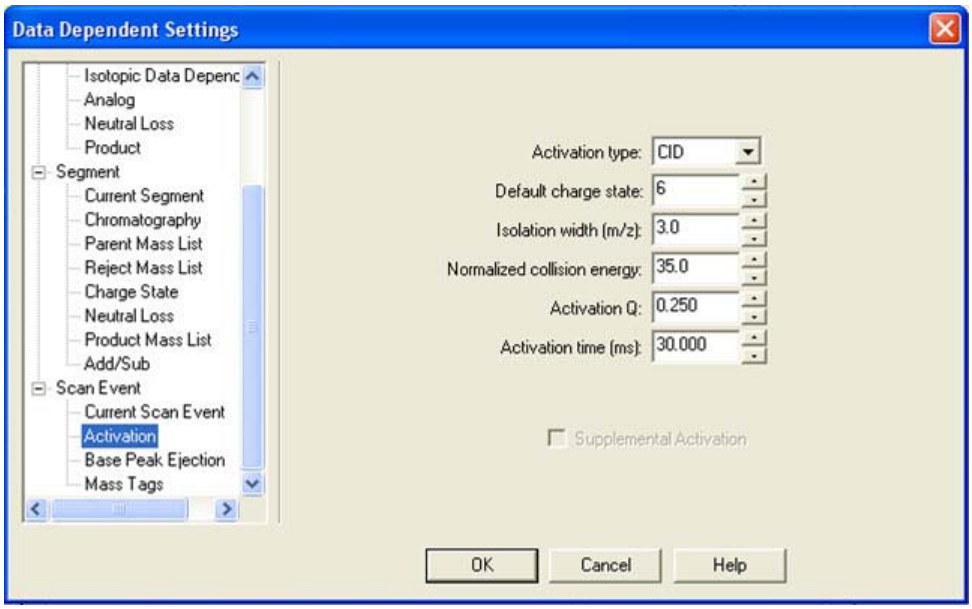

Figure 23.

17. Enable the stepped collion energy feature. Go to the LTQ Orbitrap XL → Stepped Collision energy tab. Check the Enabled box and enter a Collision Energy Width of 10 % and for Number of steps a value of 2.

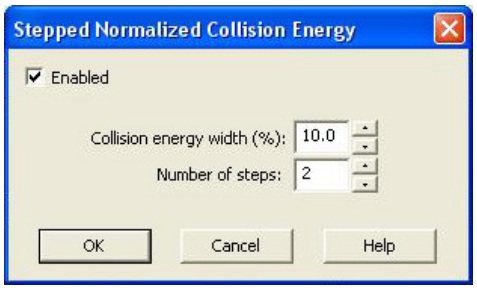

Figure 24.

18. Once the method setup is complete, click on the Summary tab, located at the top right portion of the screen. The summary of the method you have just created will be similar to that which is shown in Figures 25 and 26.

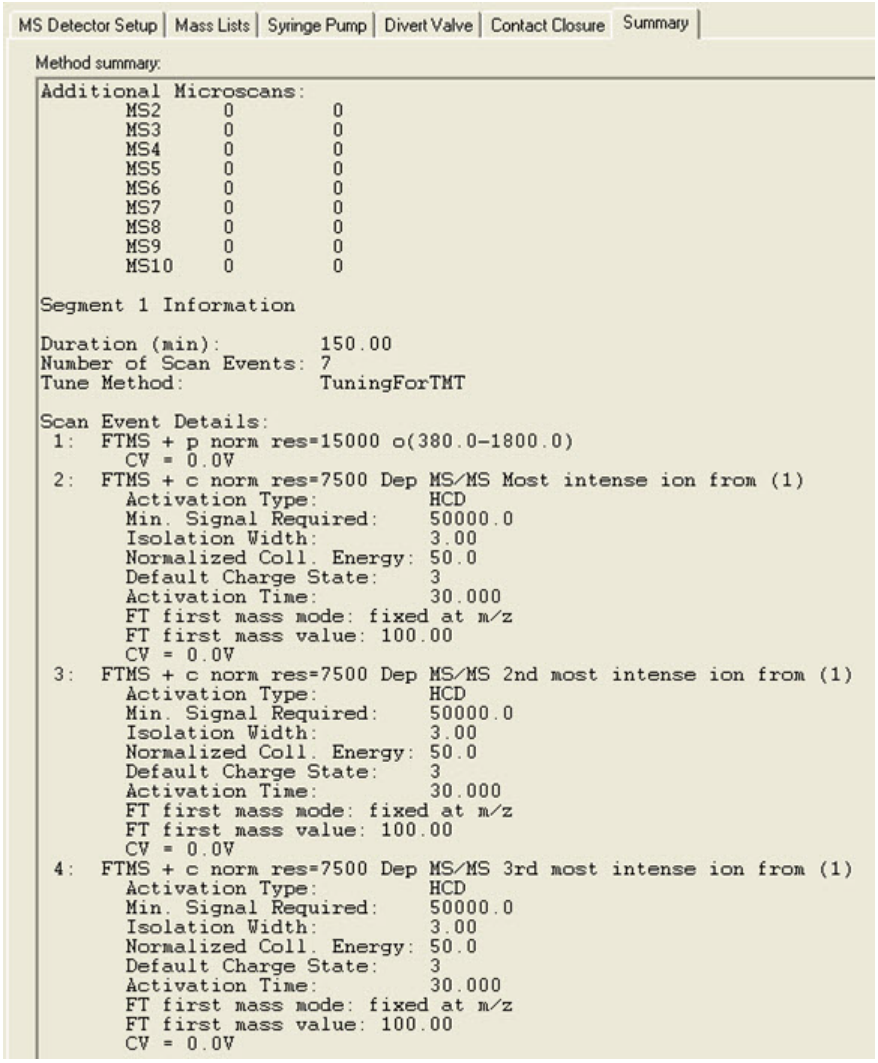

Figure 25.

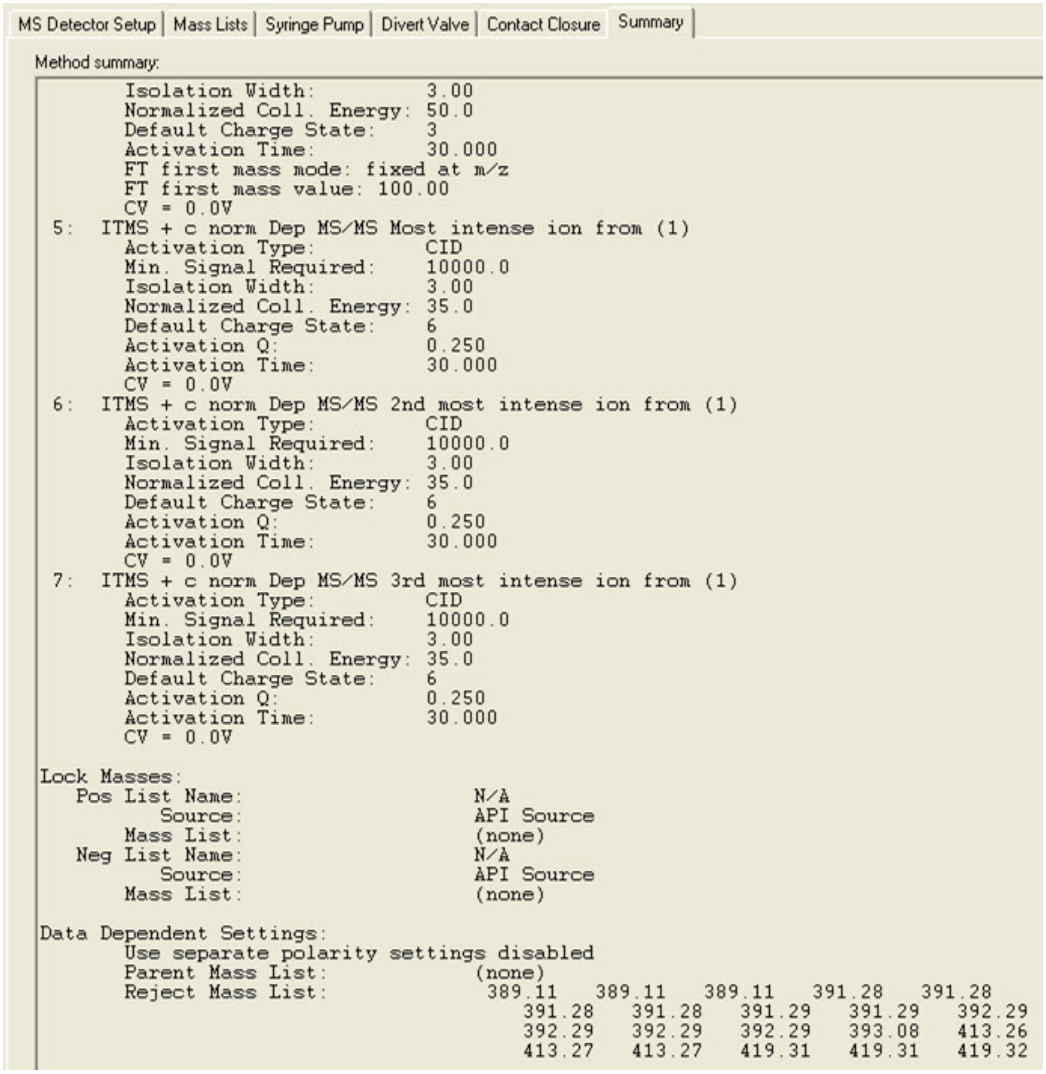

Figure 26.

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