How to setup a PRM assay on the QExactive Plus (SW v 2.7)

Inclusion List

First you need a list of peptides representing the proteins you want to monitor/quantify. You can use prior DDA data, databases or theoretical peptides.
Generate an inclusion list including:

- Mono-isotopic precursor m/z
- charge state (z)
- For scheduling you'll need the start and end times,
- you can also include the peptide sequence in the comment field if you wish

You can use skyline to generate the list for you. Follow their tutorials. Note you'll likely use Skyline to analyze your data, so you might as well use it to generate your inclusion list.
https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view?name=tutorial_targeted_msms

Instrument method

Open the method editor, expand the "*Global Lists*" and click on "Inclusion List". The "*Method editor – Inclusion List*" will pop up.
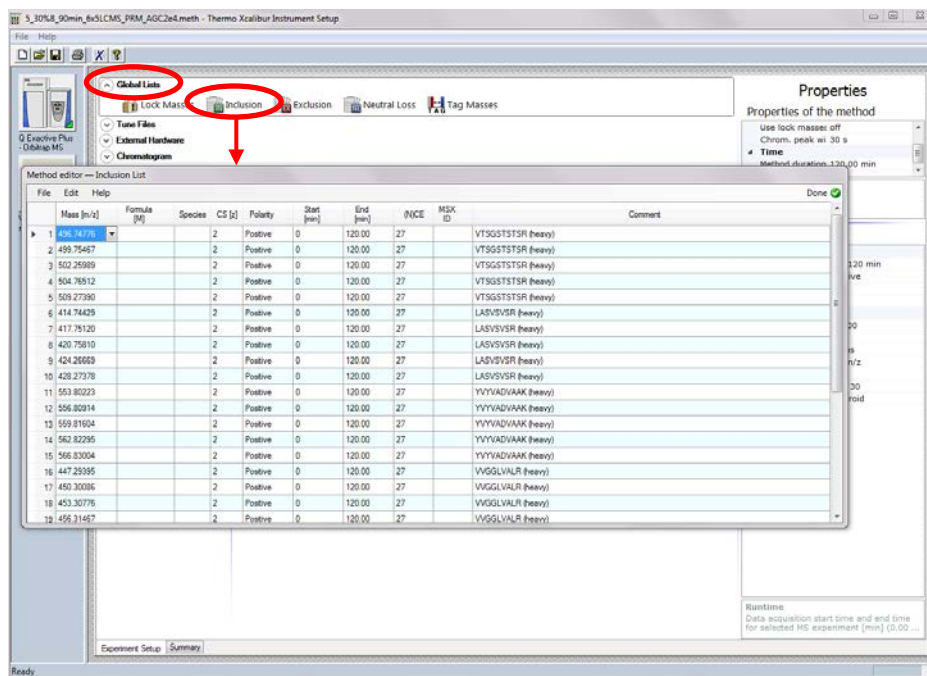
You can enter all the values manually or you can export a template to csv and copy/paste your list.
To export a template, enter a couple of values (I usually enter the m/z and z in the first row only).
Then click on File/Export and give it a path and file name, this will save the template as a csv file.
Now open that file in Excel:

Now copy your inclusion list into this file, save and close it. Note you need to close the csv file in order to import into the method editor.
You'll need to enter monoisotopic masses for the peptides of interest and the charge state and polarity. If you want to schedule the monitoring enter a start and end time as well, if left blank the targets will be monitored the entire time of the acquisition. NCE can be set in the list or globally. In the comment field I usually enter the peptide sequence.

Go back to "*Method editor – Inclusion List*" click File/Import (make sure the csv file is closed)
Select the csv file and hit ok. You should now see all the values for your inclusion list.
Click the green checkmark labeled done to safe the list. Note any of the instrument modules that use the inclusion list will use this list (PRM, DIA, targeted SIM etc.)

Instrument paramters

Now you need to setup your method parameters:
This is a screen shot of a common PRM method we use.

Make sure the user role (upper right corner, under global settings) is set to Advanced so you see all the parameters you can change.

- We've been using the default tolerances, but you can customize them if you wish.
- Resolution: to achieve the fastest scan speed, choose the lowest available resolution. Note you'll need to know the resolution setting you use to acquire the data when you go to import data into skyline.
- AGC target: we typically use 2e4-1e5
- Maximum IT (ion time): 50 ms at 17500 resolution, you can increase the ion time but it will slow down the instrument. Note in targeted experiments when the targeted precursor is absent, the instrument will fill to the max IT, because there are not enough ions to reach the target value. Only when ions are present will the AGC start regulating the IT. In other words the IT is lower than the max IT only if your target peptide is eluting. Because the instrument is doing the fill while it is acquiring data in the Orbitrap, you have "free fill time". So you don't waste time as long as your IT does not exceed the OT scan time.
- Isolation width: on the QE we used 2m/z, on the QE plus we use 1.6 m/z, the lower you go the fewer contaminating peptides are co-isolated, but at some point the sensitivity starts to drop.
 You can try using an offset, to try and get all/more of the isotopes included:
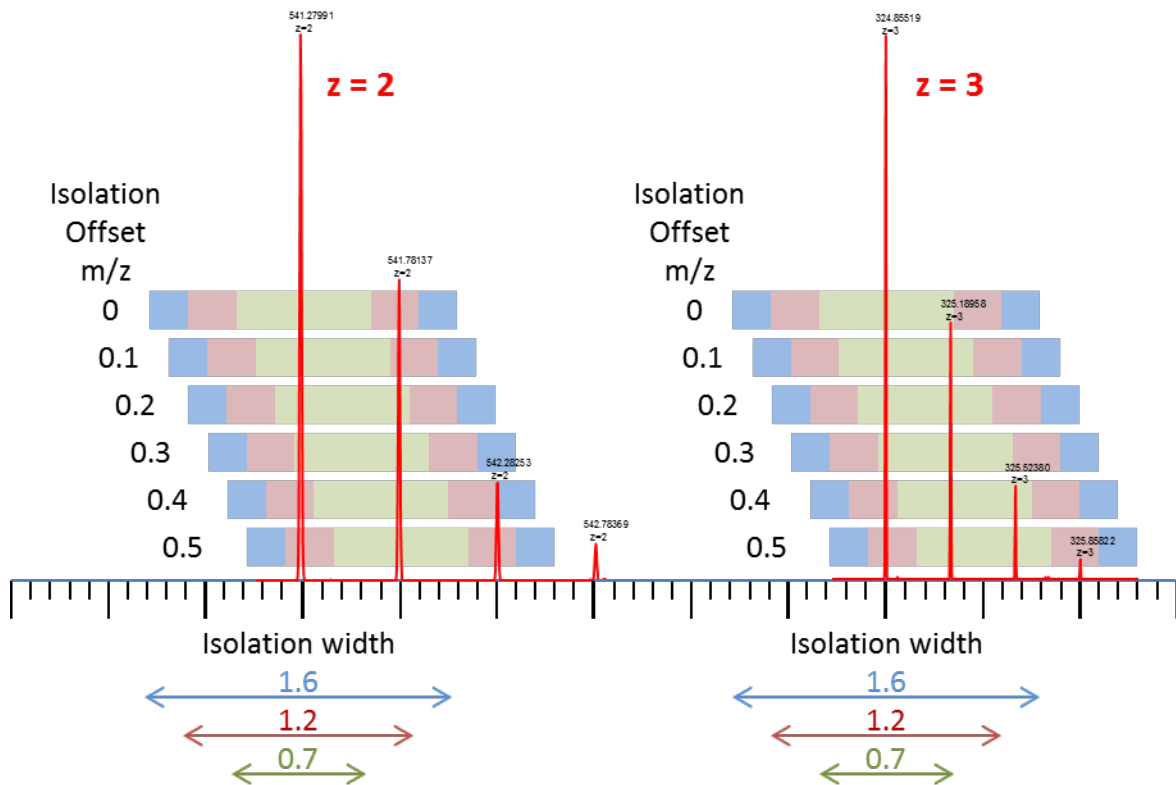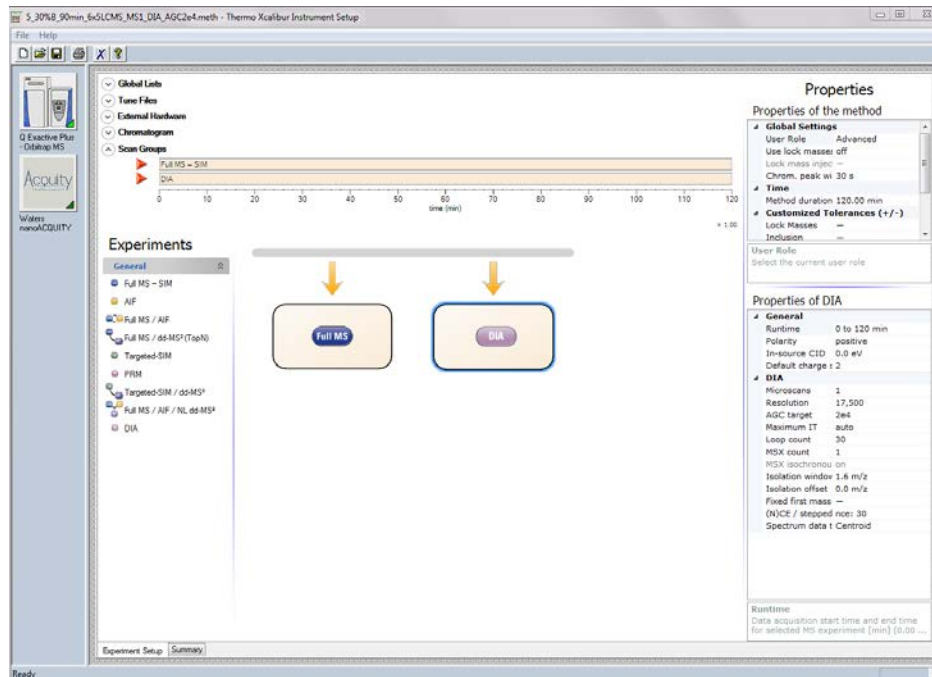
Full Scan - PRM

If you wish to acquire a full scan as well you can include the "*Full MS – SIM*" Experiment:

To include a Full scan in between the PRM scans, we tried setting it up like this, but unfortunately it alternates between full scan and a single PRM scan, rather than one full scan followed but all the PRM scans.
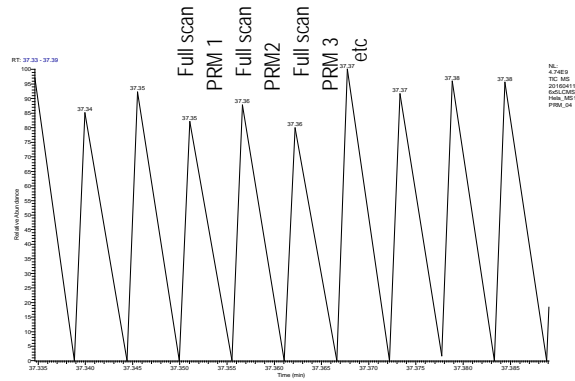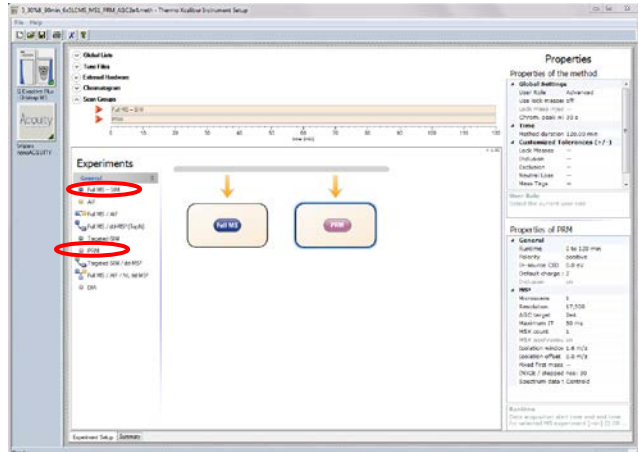
That's likely a bug that may get fixed in future SW version.

To get around that we use the DIA experiment instead of the PRM (but using the PRM inclusion list) like this:


With this setup it will do a full scan, and then go through all the precursors in your list (during the scheduled time). You can also define the loop count, e.g. 5, in that case it will do a full scan followed by the first 5 precursor (1-5) MS/MS defined in your list, then a full scan followed by the next 5 precursors (6-10) in the list, etc. (see screen shots below).

Note if you set the "Maximum IT" to 'auto' it will max out at 55 ms, in other words it will never exceed 55 ms, in our tests the scan speed with max IT set to "auto" was 11 Hz and with fixed IT at 50 ms 11.6 Hz.
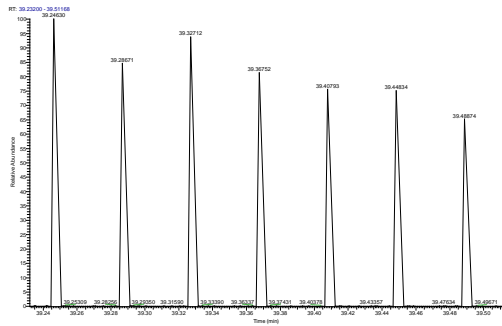
30 PRM inclusion masses, Loop count 30
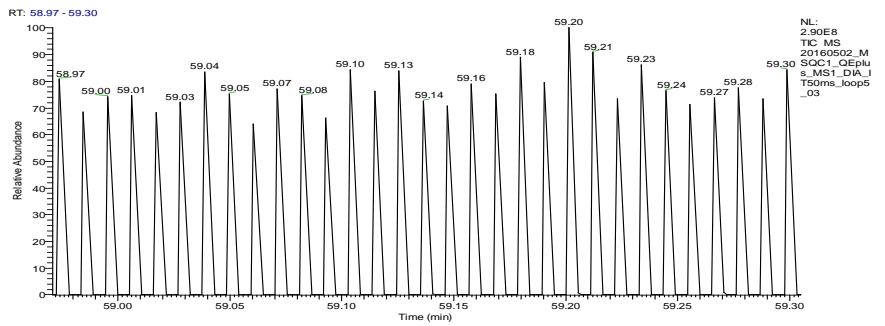
30 PRM inclusion masses, loop count 5

We also tried the Full-ddMS2 Experiment using the inclusion list and not allowing to pick others but that didn't work at all as it only did MS2 if the precursor exceeds a certain threshold. But that didn't really work for our experiments.

How many peptides can be monitored?

Max scan speed with 17500 resolution is 13 Hz (according to Thermo), so you can monitor up to 13 peptides per second. If the chromatographic peak width is 30 seconds and you want to monitor a peptide about 10 times across the chromatographic elution, your total cycle time should be about 3 seconds (30/10). So 13 Hz * 3 seconds = 39 peptides at any given time.

Chrom. Peak width [sec]		Points across the peak		Cycle time [sec]		Scan speed (depends on resolution) [Hz]		# of peptides
30	/	5	=	6	*	13	=	78
30	/	10	=	3	*	13	=	39
30	/	15	=	2	*	13	=	26

To monitor more peptides you need to schedule them using the start and end times in the inclusion list.

The table below contains approximate scan rate for the different resolution settings:

Fusion/Lumos			Transient length [ms]	*Q Exactive*		
Resolution at m/z 200	"Free" injection time [ms]	Approx. scan speed [Hz]		Resolution at m/z 200	"Free" injection time [ms]	Approx. scan speed [Hz]
15 000	22	18/20	32			
30 000	54	15	64	17 500	50	13
60 000	118	7.5	128	35 000	110	7
120 000	246	4	256	70 000	240	3
240 000	502	2	512	140 000	500	1.5
			1064	280 000	1000	<1
>450 000	1014	<1	1024			

You can check the scan speed for your particular method, just open the raw file in qual browser and zoom in, if you acquired a full scan just look at the time difference between full scans and calculate the scan speed base on the number of scans you had setup to run (full scan plus number of PRM/DIA scans):
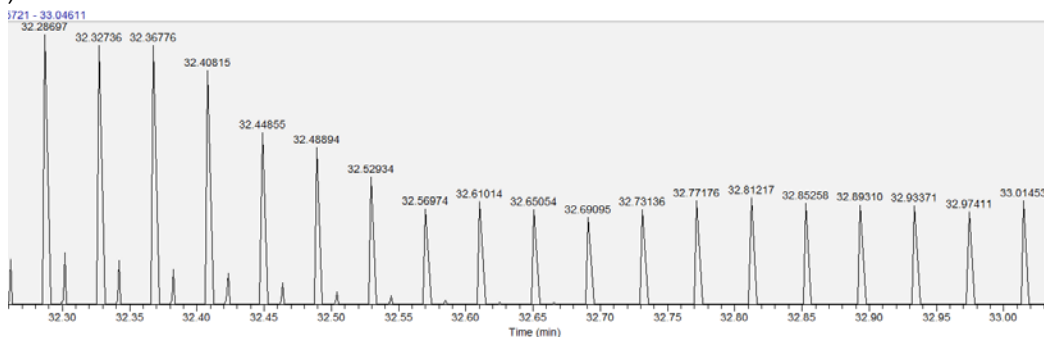
Now calculate the time difference between the full scans and calculate the scan speed based on the number of MS2 scans between the full scans. In this example is about 12.8 Hz (our inclusion list has 30 precursor masses plus one full scan).