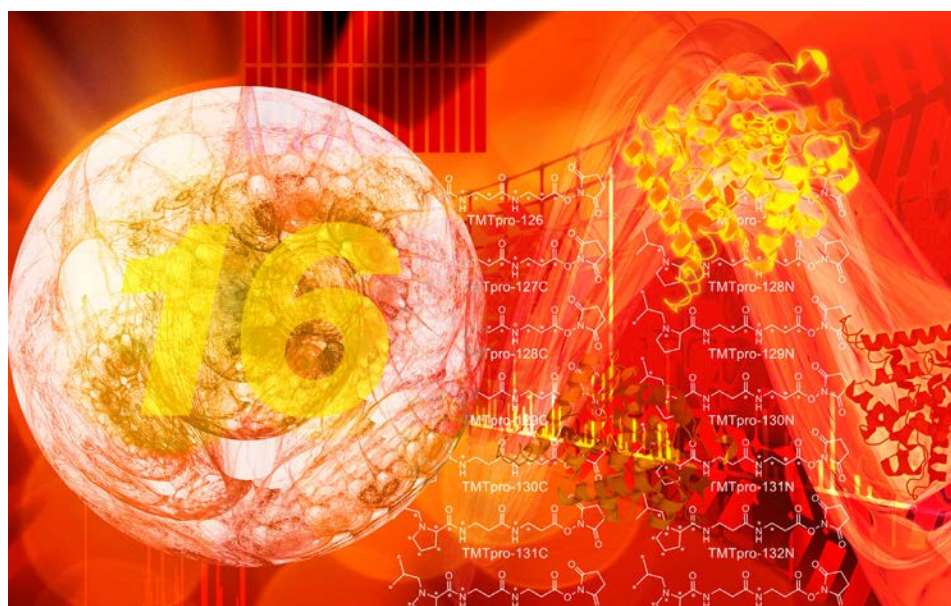
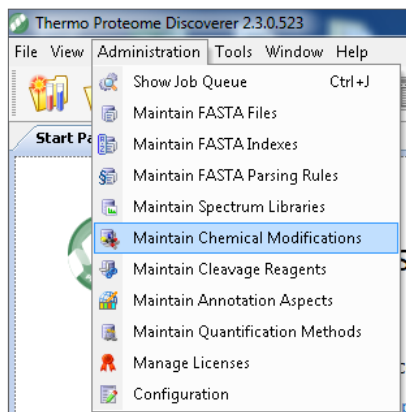


# Using TMTpro 16plex reagents with Proteome Discoverer 2.3 software

## Creating modifications for Thermo Scientific™ TMTpro™ reagents

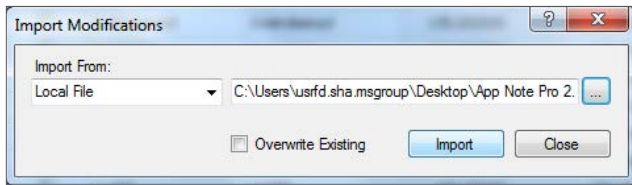
- Starting at the home screen of Thermo Scientific™ Proteome Discoverer™ 2.3 software, select “Maintain Chemical Modifications” under the “Administration” tab.



- Import the file “Modifications” using the “Import” tab.

Is Active	Modification	Abbreviation	Delta Mass	Delta Average Mass	Substitution	Leaving Group	Position	Unimod Accession No.
<input checked="" type="checkbox"/>	TMT	TMT	224.152478	224.2994	H(20) C(12) N(2) O(2)		Protein_N_Terminus	739
<input checked="" type="checkbox"/>	TMT	TMT	224.152478	224.2994	H(20) C(12) N(2) O(2)		Any_N_Terminus	739
<input checked="" type="checkbox"/>	TMT	TMT	224.152478	224.2994	H(20) C(12) N(2) O(2)		Any	739
<input checked="" type="checkbox"/>	TMT2plex	TMT2plex	225.155833	225.2921	H(20) C(11) 13C N(2) O(2)		Any	738
<input checked="" type="checkbox"/>	TMT2plex	TMT2plex	225.155833	225.2921	H(20) C(11) 13C N(2) O(2)		Protein_N_Terminus	738
<input checked="" type="checkbox"/>	TMT2plex	TMT2plex	225.155833	225.2921	H(20) C(11) 13C N(2) O(2)		Any_N_Terminus	738
<input checked="" type="checkbox"/>	TMT6plex	TMT6plex	229.162932	229.2634	H(20) C(8) 13C(4) N 15N O(2)		Any	737
<input checked="" type="checkbox"/>	TMT6plex	TMT6plex	229.162932	229.2634	H(20) C(8) 13C(4) N 15N O(2)		Protein_N_Terminus	737

- Select “Local File” in the “Import From” field and browse for the modification file. Select the file and click “Import”.



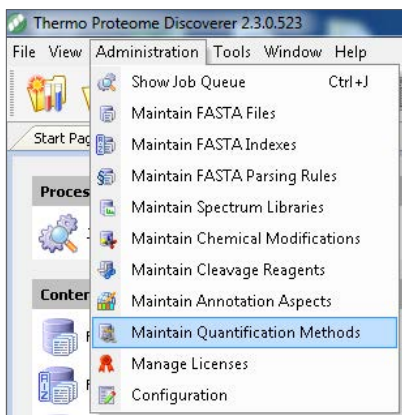
- If an error occurs, check for an existing modification with the name “TMTpro”, and rename or remove if present. Click “Apply” before importing the modification file. It may be necessary to close and reopen Proteome Discoverer software to implement the changes.
- The modifications should appear in the window as shown below:

Is Active	Modification	Abbreviation	Delta Mass	Delta Average Mass	Substitution	Leaving Group	Position	Unimod Accession No.
<input checked="" type="checkbox"/>	TMTpro		=	=	A	A	A	=
* Add a Modification...								
<input checked="" type="checkbox"/>	TMTpro 0	TMTpro 0	295.18959	295.3773	H(25) C(15) N(3...		Any_N_Te...	-1
<input checked="" type="checkbox"/>	TMTpro 0	TMTpro 0	295.1896	295.1896	H(25) C(15) N(3...		Protein_N...	0
<input checked="" type="checkbox"/>	TMTpro	TMTpro	304.2071...	304.3127	H(25) C(8) 13C(...		Any	-1
Amino Acid Name			One Letter Code	Classification				
Lysine			K	Isotopic label				
* Add an Amino Acid Site...								
Is Active	Modification	Abbreviation	Delta Mass	Delta Average Mass	Substitution	Leaving Group	Position	Unimod Accession No.
<input checked="" type="checkbox"/>	TMTpro	TMTpro	304.2071...	304.3127	H(25) C(8) 1...		Any_N_Te...	-1
Amino Acid Name			One Letter Code	Classification				
N-Terminus				Isotopic label				
* Add an Amino Acid Site...								

- Click “Apply” after implementing any changes to the modifications table. It may be necessary to close and reopen Proteome Discoverer software to implement the changes.

## Creating a TMTpro 16 plex quantification method

- Under the “Administration” tab, select “Maintain Quantification Methods”.



- Import the file "TMTpro 16plex lotUH290428.method" using the "Import" tab.

Status	Method Name	Description	Is Active
✓	Dimethylation 3plex (C2H4, C2D4, 13C2D4)	Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method	✓
✓	Full 18O Labeling (O2   O18O)	18O labeling method for fully labeled samples	✓
✓	Incomplete 18O Labeling (O2   O18O + 18O2)	18O labeling method for incompletely labeled samples	✓
✓	Iodo TMT 6plex	Method for cysteine-reactive 6-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	ITRAQ 4plex	Method for ITRAQ™ 4-plex mass tags by Applied Biosystems	✓
✓	ITRAQ 8plex	Method for ITRAQ™ 8-plex mass tags by Applied Biosystems	✓
✓	Low Resolution Iodo TMT 6plex	Method for low resolution cysteine-reactive 6-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	Low Resolution TMTe 6plex	Method for low resolution 6-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	SILAC 2plex (Arg10, Lys6)	SILAC 2plex (Arg10, Lys6) Method	✓
✓	SILAC 2plex (Arg10, Lys8)	SILAC 2plex (Arg10, Lys8) Method	✓
✓	SILAC 2plex (Ile6)	SILAC 2plex (Ile6) Method	✓
✓	SILAC 3plex (Arg6, Lys4   Arg10, Lys8)	SILAC 3plex (Arg6, Lys4   Arg10, Lys8) Method	✓
✓	SILAC 3plex (Arg6, Lys6   Arg10, Lys8)	SILAC 3plex (Arg6, Lys6   Arg10, Lys8) Method	✓

- The following window should appear:

Quantification Method Editor: TMTpro 16plex lotUH290428

Quan Channels

Residue Modification: TMTpro / +304.207 Da K

N-Terminal Modification: TMTpro / +304.207 Da

Reporter Ion Isotopic Distribution Correction Factors

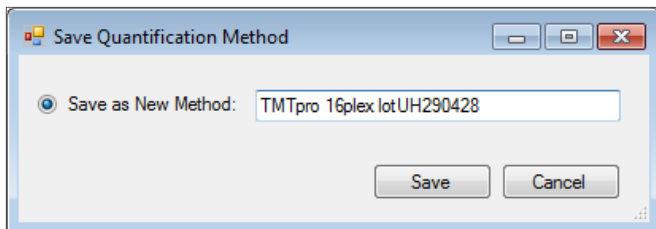
Mass Tag	Reporter Ion Mass	- 2	Affects	- 1	Affects	Main	+ 1	Affects	+ 2	Affects	Active
126	126.12772	0	N/A	0	N/A	100	7.73	127C	0	N/A	Used
127N	127.12476	0	N/A	0	N/A	100	7.46	128N	0	N/A	Used
127C	127.13108	0	N/A	0.71	126	100	6.62	128C	0	N/A	Used
128N	128.12811	0	N/A	0.75	127N	100	6.67	129N	0	N/A	Used
128C	128.13443	0	N/A	1.34	127C	100	5.31	129C	0	N/A	Used
129N	129.13147	0	N/A	1.29	128N	100	5.48	130N	0	N/A	Used
129C	129.13779	0	N/A	2.34	128C	100	4.87	130C	0	N/A	Used
130N	130.13482	0	N/A	2.36	129N	100	4.57	131N	0	N/A	Used
130C	130.14114	0	N/A	2.67	129C	100	3.85	131C	0	N/A	Used
131N	131.13818	0	N/A	2.71	130N	100	3.73	132N	0	N/A	Used
131C	131.14445	0	N/A	3.69	130C	100	2.77	132C	0	N/A	Used
132N	132.14153	0	N/A	2.51	131N	100	2.76	133N	0	N/A	Used
132C	132.14785	0	N/A	4.11	131C	100	1.63	133C	0	N/A	Used
133N	133.14489	0	N/A	3.09	132N	100	1.58	134N	0	N/A	Used
133C	133.15121	0	N/A	4.63	132C	100	0.88	N/A	0	N/A	Used
134N	134.14824	0	N/A	4.82	133N	100	0.86	N/A	0	N/A	Used

Reporter quan method, only correction factors can be edited

OK Cancel Help

**Note:** Each TMTpro quantification method contains prefilled correction factors that are specific for each lot of tags. An example is shown for lot UH290428.

10. Click “OK” and designate a file name if desired. Save as a new method.



11. A correction factors file should now be visible in the list of quantification methods.

12. Proceed with data analysis of TMTpro RAW files.

Find out more at [thermofisher.com/tmtpro](https://thermofisher.com/tmtpro)