

Biotherapeutic subunit mass analysis

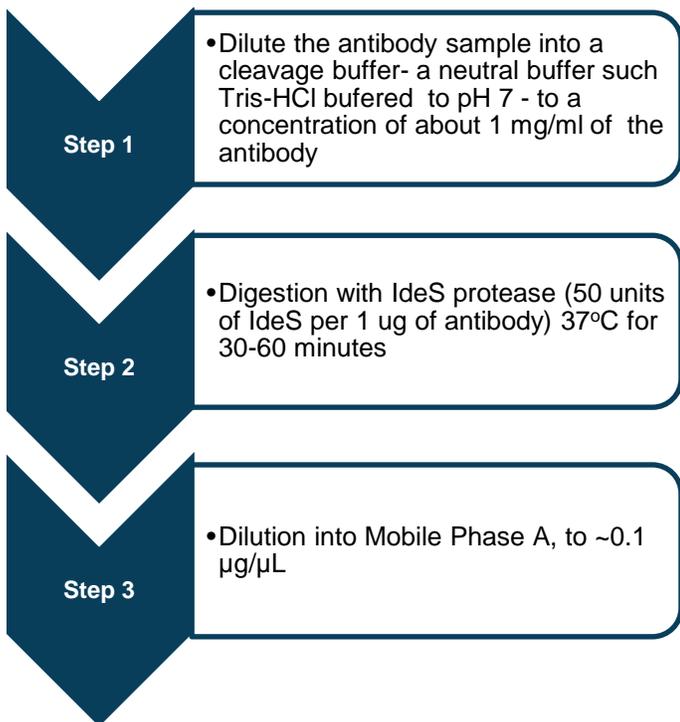
Routine high-resolution accurate mass analysis of biotherapeutic subunits on the X500B QTOF System

Method details for the routine characterization of trastuzumab biotherapeutic protein following an IdeS subunit digestion strategy. Analysis performed by high-resolution accurate mass detection using HPLC coupled with the X500B QTOF System, powered by SCIEX OS Software.



Sample Prep

A generic sample preparation strategy is shown for IdeS subunit digestion and clean-up of an intact biotherapeutic prior to LC-MS analysis.



LC Method

<i>Column</i>	Agilent Zorbax SB300 C-8 1mm X 75mm	
<i>Mobile Phase A</i>	Water, 0.1% Formic acid	
<i>Mobile Phase B</i>	Acetonitrile, 0.1% Formic acid	
<i>Flow rate</i>	200 µL/min	
<i>Column temperature</i>	80 ^o C	
<i>Injection volume</i>	10 µL, ~1 µg total protein	
<i>Gradient profile</i>	Time (min)	% B
	2.0	25
	6.0	60
	7.0	60
	7.1	80
	9.0	80
	9.5	25
	10.5	25

MS Method

Suggested starting MS method parameters for routine mAb subunit analysis as displayed in SCIEX OS. For best sensitivity and resolution, the declustering potential (DP) and collision energy (CE) parameters should be optimized for each individual biotherapeutic.

The screenshot shows the 'Subunit protein analysis MS' configuration window in SCIEX OS. The interface includes a top navigation bar with 'New', 'Open...', 'Save', 'Print...', and 'Advanced' buttons. The main window is divided into a left sidebar and a main configuration area. The sidebar shows 'Method Overview' with details for 'Device: X500 QTOF' and 'Ion Source: TurboSpray', and a 'TOF MS' section with a time range of '0 min - 10 min'. The main configuration area is organized into several sections: 'Method Overview' (Method duration: 10 min, Total scan time: 0.527524 sec, Estimated cycles: 1137, Intact protein mode: ON, checkboxes for 'Large proteins (>70 kDa)' and 'Decrease detector voltage'), 'Source and Gas Parameters' (Ion source gas 1 and 2: 45 psi, Curtain gas: 30 psi, CAD gas: 7 psi, Temperature: 450 °C), 'Experiment' (TOF MS selected, Polarity: Positive, TOF start mass: 900 Da, TOF stop mass: 4500 Da, Accumulation time: 0.5 s, Spray voltage: 5500 V, Declustering potential: 200 V, DP spread: 0 V, Collision energy: 10 V, CE spread: 0 V), and 'Advanced Experiment Settings' (Time bins to sum: 80, Channel 1, 2, 3, and 4 checkboxes). The bottom status bar shows 'Data Acquisition' with 'MS' selected and 'Start', 'Stop', and 'Save...' buttons.

Batch

In Batch setup, open the 'Automated Calibration Editor' window in order to select the use of the autocalibration function. Designate use of the 'X500 ESI Positive Calibration Solution', and then determine how often you would like the system to perform a fast, automated calibration. These short calibrations will be added automatically to your queue once you have submitted a sample batch.

The screenshot shows the 'Batch' software interface. At the top, there is a menu bar with options like 'Auto-Calibrate...', 'Plate Layout...', 'New', 'Open', 'Save', 'Print...', 'Manage', and 'Submit'. Below the menu bar is a table with the following columns: Sample Name, MS Method, LC Method, Rack code, Vial position, and Data File. The table contains one row of data: 'Intact protein', 'intact protein analysis MS', 'Intact_10min', '1.5mL (105 vial)', '1', and 'Intact protein file'. Overlaid on the table is a dialog box titled 'Batch - Automatic Calibration Editor'. The dialog box contains the following fields: 'Ion reference table' (set to 'X500 ESI Positive Calibration Solu...'), 'Calibrate every' (set to '3' samples), 'Calibrant delivery' (a dropdown menu with 'X500 ESI Positive Calibration Solution' selected), and 'CDS channel' (set to '1'). There are 'Edit...', 'OK', and 'Cancel' buttons in the dialog box.

This is a close-up view of the 'Batch - Automatic Calibration Editor' dialog box. It shows the following settings: 'Ion reference table' is set to 'X500 ESI Positive Calibration Solu...', 'Calibrate every' is set to '3' samples, 'Calibrant delivery' is set to 'CDS', and 'CDS channel' is set to '1'. The 'Edit...' button is visible next to the 'Ion reference table' dropdown. 'OK' and 'Cancel' buttons are at the bottom right.

Data Processing

Process intact biotherapeutic data in BioPharmaView™ Software 2.0.

Input the protein sequence, and assign potential modifications in the 'Assay Information' window.

Trastuzumab subunit

Assay Information | **Sequence Features** | Intact Protein | Peptide Mapping

Protein Sequence

Protein Type: Antibody | **Add Chain** | Unmodified Protein MWs: Monoisotopic: 121496.1071 Average: 121572.61

Chain 1: LC 1 | **Delete Chain**

AA Indexes:

1-110 DIQMTQSPSSLSASVGRVITTCRASQDVNTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSRSGDFTLTISSLQPEDFATYYCQHYHTPPPTFGQGTKVEIKRRTV
 111-214 AAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Chain 2: HC nterm 1 | **Delete Chain**

AA Indexes:

1-110 EVQLVESGGGLVQPGGSLRSLSCAASGFNIKDTYIHWVRQAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDFYAMDYW
 111-220 GQGTLLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVKDKVEP
 221-239 KSCDKTHTCPPCPAPELLG

Chain 3: HC nterm 2 | **Delete Chain**

AA Indexes:

1-110 EVQLVESGGGLVQPGGSLRSLSCAASGFNIKDTYIHWVRQAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDFYAMDYW
 111-220 GQGTLLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVKDKVEP
 221-239 KSCDKTHTCPPCPAPELLG

Chain 4: LC 2 | **Delete Chain**

AA Indexes:

1-110 DIQMTQSPSSLSASVGRVITTCRASQDVNTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSRSGDFTLTISSLQPEDFATYYCQHYHTPPPTFGQGTKVEIKRRTV
 111-214 AAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Chain 5: HC cterm | **Delete Chain**

AA Indexes:

1-110 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREP
 111-211 QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYTKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKLSLSLSPGK

Modifications

Cysteine Modifications Can Replace Disulfide Bonds

Disulfide Bonds - (14) | **Import** | **Export...**

Chains	Type	Name	Position	Maximum Mods per Chain	Modified AA	Applies To	Workflow Usage	Mass Shift
1	5 Internal	dHex	61	-	N	STN	Both	146.0579
2	5 Internal	G0	61	-	N	N	Both	1298.4760
3	5 Internal	G1	61	-	N	N	Both	1460.5288
4	5 Internal	G1F	61	-	N	N	Both	1606.5867
5	5 Internal	G2	61	-	N	N	Both	1622.5816
6	5 Internal	G2F	61	-	N	N	Both	1768.6395
7	5 Internal	G0F-GlcNAc	61	-	N	N	Both	1241.4545
8	5 Internal	G1F-GlcNAc	61	-	N	N	Both	1403.5073
9	5 Internal	G0F	61	-	N	N	Both	1444.5339
10	5 C-terminal	Protein Terminal Lys-los	-	-	K	K	Both	-128.0950

From Chain	To Chain	From Cysteine	To Cysteine
1	1	1	23
2	1	1	134
3	1	2	214
4	2	2	22
5	2	2	147
6	5	5	25
7	5	5	131
8	4	4	23
9	4	4	134
10	4	3	214
11	3	3	22
12	3	3	147
13	2	3	229
14	2	3	232

Add modifications... | **Delete selected modifications** | **Edit bond...** | **Add bonds...** | **Delete selected bonds**

Pharma and Biopharma



Navigate to the 'Intact Protein' tab complete processing parameters and to generate the protein forms for matching.

The screenshot displays the BioPharmaView software interface for the 'Trastuzumab subunit' project. The 'Intact Protein' tab is selected, showing various processing parameters and a table of characterized proteins.

Processing Parameters:

- Matching Tolerance: ± 5.00 Da
- Start m/z: 600.00
- Stop m/z: 3000.00
- Start Mass: 18786.94 Da
- Stop Mass: 102629.28 Da
- RT Range Processing: Time Selection
- Perform LC Peak Detection:
- Start RT: 3.93 min
- Stop RT: 4.98 min

Batch Processing Parameters:

- Retention Time Tolerance: ± 1.00 min

Batch Processing Pass / Fail Criteria:

- Reconstruction Area Limits: ± 10.0 %
- Required Form Minimum: ≥ 80 %
- Restricted Form Maximum: ≤ 0 %

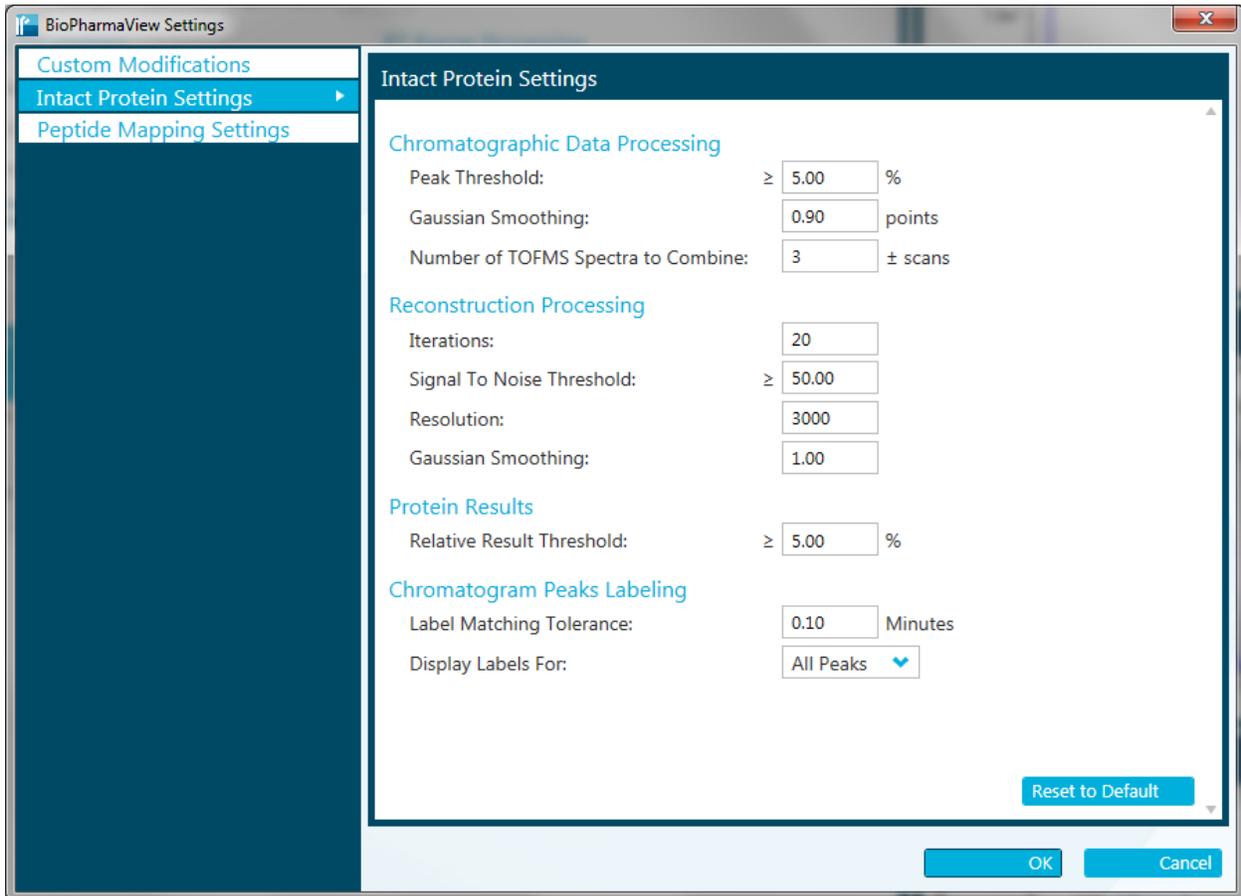
Maximum Number of Combined Modifications per Protein:

Characterized Proteins Reduced Protein Form

Batch Usage	Protein Name	Modifications	User Defined	Mono. Mass	Avg. Mass	Match...	Reconstruction Area	Retention Time
1 Optional	Trastuzumab-HC cterm	Protein Terminal Lys-loss - 1		23771.8983	23786.94		-	-
2 Optional	Trastuzumab-HC cterm			23899.9932	23915.11		-	-
3 Optional	Trastuzumab-HC cterm	dHex - 1 Protein Terminal Lys-loss - 1		23917.9562	23933.08		-	-
4 Optional	Trastuzumab-HC cterm	dHex - 1		24046.0511	24061.25		-	-
5 Optional	Trastuzumab-HC cterm	G0F-GlcNAc - 1 Protein Terminal Lys-loss - 1		25013.3527	25029.09		-	-
6 Optional	Trastuzumab-HC cterm	G0 - 1 Protein Terminal Lys-loss - 1		25070.3742	25086.14		-	-
7 Optional	Trastuzumab-HC cterm	G0F-GlcNAc - 1		25141.4477	25157.26		-	-
8 Optional	Trastuzumab-HC cterm	G1F-GlcNAc - 1 Protein Terminal Lys-loss - 1		25175.4056	25191.23		-	-
9 Optional	Trastuzumab-HC cterm	G0 - 1		25198.4692	25214.32		-	-
10 Optional	Trastuzumab-HC cterm	G0F - 1 Protein Terminal Lys-loss - 1		25216.4321	25232.28		-	-
11 Optional	Trastuzumab-HC cterm	G1 - 1 Protein Terminal Lys-loss - 1		25232.4270	25248.28		-	-
12 Optional	Trastuzumab-HC cterm	G1F-GlcNAc - 1		25303.5005	25319.41		-	-

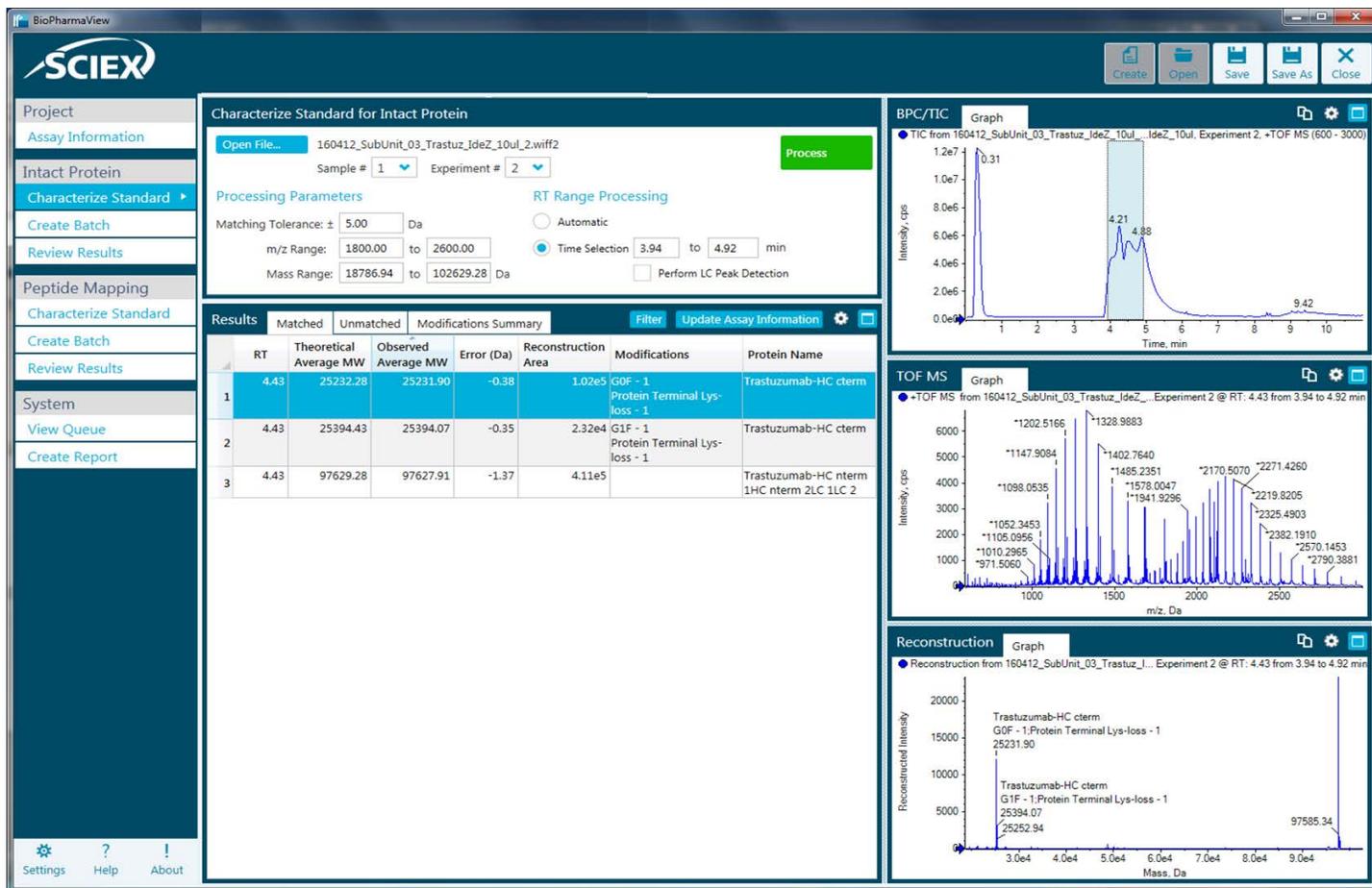
Buttons: Settings, Help, About, Import, Delete, Reset Characterized Proteins

Navigate to the 'Settings' icon and review your global 'Intact Protein Settings'



Pharma and Biopharma

Protein deconvolution of each subunit can be performed in seconds, on either a single datafile, or on multiple samples using the batch processing function. Below is shown the deconvolution results for the Fc domain of the biotherapeutic, as well as the Fab2 domain of the biotherapeutic (non-reduced).



[Learn more at sciex.com/X500B](http://sciex.com/X500B)

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