

Biotherapeutic subunit mass analysis

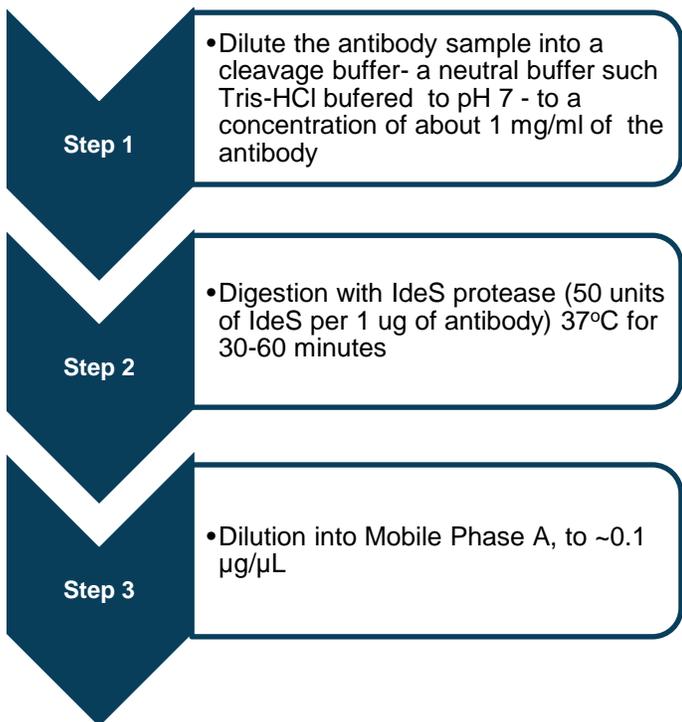
Routine high-resolution accurate mass analysis of biotherapeutic subunits on the X500B QTOF System

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 area is divided into several sections:

- Method Overview:** Shows 'Device: X500 QTOF' and 'Ion Source: TurboSpray'. A 'TOF MS' tab is selected, showing a time range of '0 min - 10 min'.
- General Parameters:** Method duration is 10 min, Total scan time is 0.527524 sec, Estimated cycles is 1137, and Intact protein mode is ON. There are checkboxes for 'Large proteins (>70 kDa)' (checked) and 'Decrease detector voltage' (unchecked).
- Source and Gas Parameters:** Ion source gas 1 and 2 are both set to 45 psi. Curtain gas is 30 psi and CAD gas is 7 psi. Temperature is 450 °C.
- Experiment (TOF MS):** Polarity is Positive. TOF start mass is 900 Da and TOF stop mass is 4500 Da. Accumulation time is 0.5 s. Spray voltage is 5500 V. Declustering potential is 200 V and DP spread is 0 V. Collision energy is 10 V and CE spread is 0 V.
- Advanced Experiment Settings:** Time bins to sum is 80. Channel 1, 3, and 4 are checked, while Channel 2 is unchecked.

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 is a table with the following columns: Sample Name, MS Method, LC Method, Rack code, Vial position, and Data File. The first row contains: Intact protein, intact protein analysis MS, Intact_10min, 1.5mL (105 vial), 1, and Intact protein file. An 'Untitled' window is open, and a 'Batch - Automatic Calibration Editor' dialog box is displayed over it. The dialog box contains the following fields and options:

- Ion reference table: X500 ESI Positive Calibration Solu... (dropdown)
- Calibrate every: 3 samples (spin box)
- Calibrant delivery: CDS (dropdown)
- CDS channel: 1 (dropdown)
- Buttons: Edit..., OK, Cancel

This is a close-up view of the 'Batch - Automatic Calibration Editor' dialog box. It shows the following configuration:

- Ion reference table: X500 ESI Positive Calibration Solu... (dropdown)
- Calibrate every: 3 samples (spin box)
- Calibrant delivery: CDS (dropdown)
- CDS channel: 1 (dropdown)
- Buttons: OK, Cancel

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 DIQMTQSPSSLSASVGRVITTCRASQDVNTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSRSGDFTLTISSLPEDFATYYCQHYHTPPPTFGQGTKVEIKRRTV
 111-214 AAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

AA Indexes:

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

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

AA Indexes:

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

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

AA Indexes:

1-110 DIQMTQSPSSLSASVGRVITTCRASQDVNTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSRSGDFTLTISSLPEDFATYYCQHYHTPPPTFGQGTKVEIKRRTV
 111-214 AAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

AA Indexes:

1-110 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREP
 111-211 QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYTKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFKCSVMHEALHNHYTQKLSLSLSPGK

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 %

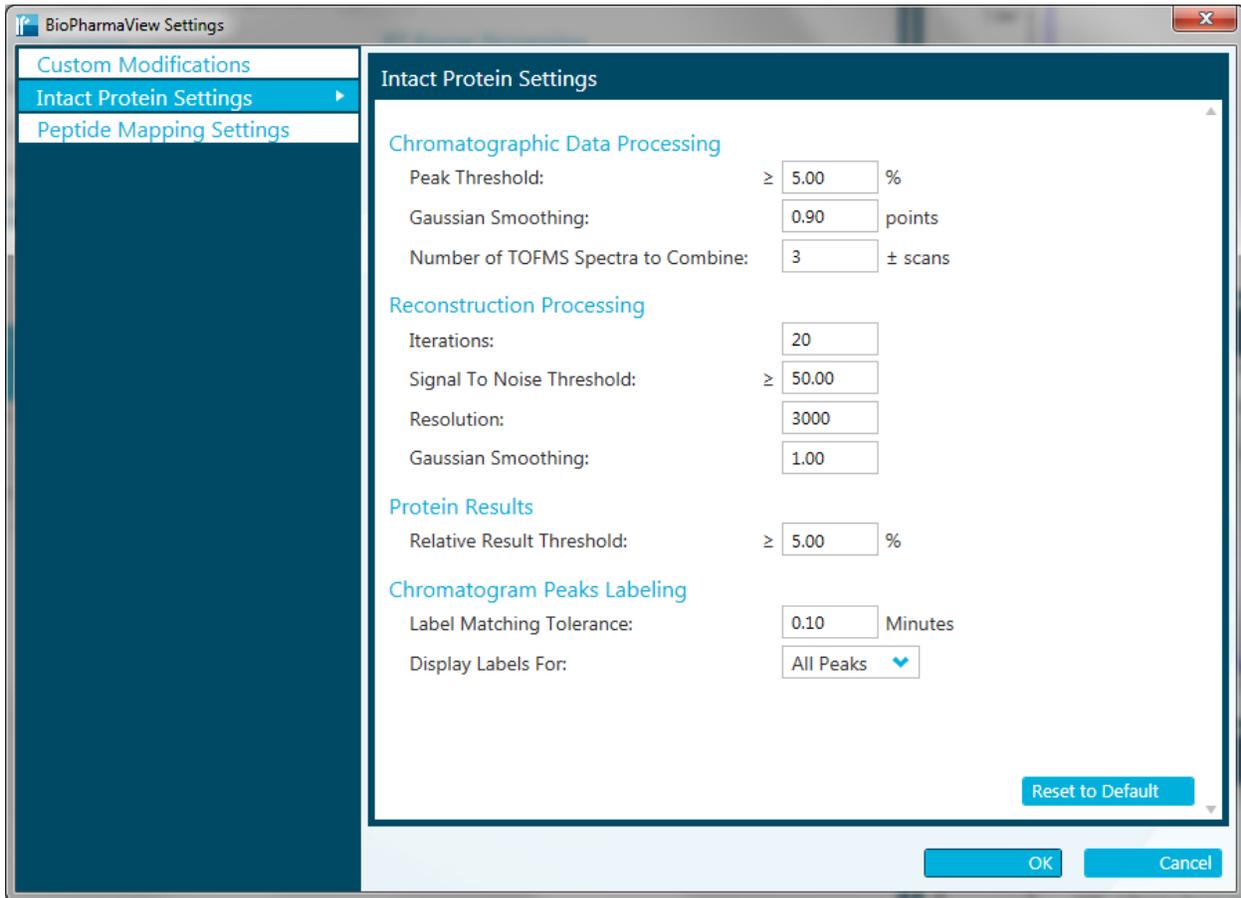
Maximum Number of Combined Modifications per Protein:

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).

The screenshot displays the BioPharmaView software interface. The main window is titled "Characterize Standard for Intact Protein". It shows the following details:

- File:** 160412_SubUnit_03_Trastuz_IdeZ_10uL_2.wiff2
- Sample #:** 1, **Experiment #:** 2
- Processing Parameters:**
 - Matching Tolerance: ± 5.00 Da
 - m/z Range: 1800.00 to 2600.00
 - Mass Range: 18786.94 to 102629.28 Da
 - RT Range Processing: Time Selection 3.94 to 4.92 min

The **Results** table is as follows:

RT	Theoretical Average MW	Observed Average MW	Error (Da)	Reconstruction Area	Modifications	Protein Name
1	4.43	25232.28	25231.90	-0.38	1.02e5 G0F - 1 Protein Terminal Lys-loss - 1	Trastuzumab-HC cterm
2	4.43	25394.43	25394.07	-0.35	2.32e4 G1F - 1 Protein Terminal Lys-loss - 1	Trastuzumab-HC cterm
3	4.43	97629.28	97627.91	-1.37	4.11e5	Trastuzumab-HC nterm 1HC nterm 2LC 1LC 2

Three graphs are displayed on the right side of the interface:

- BPC/TIC Graph:** Total Ion Chromatogram showing peaks at 0.31, 4.21, 4.88, and 9.42 minutes.
- TOF MS Graph:** Mass spectrum showing relative intensity versus m/z. Key peaks are labeled with their m/z values, including 1105.0956, 1052.3453, 1098.0535, 1147.9084, 1202.5166, 1328.9883, 1402.7640, 1485.2351, 1578.0047, 1941.9296, 2170.5070, 2271.4260, 2325.4903, 2382.1910, 2570.1453, and 2790.3881.
- Reconstruction Graph:** Shows the reconstructed intensity for the identified protein variants. Peaks are labeled with their m/z values: 25252.94, 25394.07, 25231.90, and 97585.34.

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

AB Sciex is doing business as SCIEX.

© 2016 AB Sciex. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Document number: RUO-MKT-02-4523-A