

Biotherapeutic Peptide Mapping Information Dependent Acquisition (IDA) Method

Routine biotherapeutic accurate mass peptide mapping analysis on the X500B QTOF System

Method details for the routine peptide mapping of a biotherapeutic monoclonal antibody (mAb) protein by high-resolution accurate mass analysis on the X500B QTOF System, powered by SCIEX OS Software. An information dependent acquisition (IDA) method was employed to acquire highresolution MS and MS/MS level data on the digested biologic protein product.



Sample Prep

A generic sample preparation strategy is shown for reduction and tryptic digestion of an antibody biotherapeutic prior to LC-MS analysis.





LC Method

Column	Waters Acquity UPLC BEH C18 (Column, 130 1.7 μm, 2.1 mm X 100 mm
Mobile Phase A	Water, 0.1% Formic acid	
Mobile Phase B	Acetonitrile, 0.1% Formic acid	
Flow rate	200 µL/min	
Column temperature	40° C	
Injection volume	10 μL, 1 μg total protein	
Gradient profile	Time (min)	% B
	8.0	2
	40.0	30
	60.0	50
	62.0	90
	66.0	90
	66.5	2
	75.0	2



MS Method

Suggested starting MS and MS/MS method parameters for routine peptide mapping analysis as displayed in SCIEX OS user interface. The information dependent acquisition (IDA) method criteria is shown for selecting the top 15 precursor ions for high-resolution MS/MS in each cycle. For best sequence coverage and sensitivity, the specific IDA criteria parameters should be optimized for each individual biotherapeutic and HPLC separation used.

ြ Peptide Map_IDA_	75min						
Method Overview Device: X500 QTOF Ion Source: TurboSpray	Method duration Estimated cycles:	75 🗘 min 4719	Total scan time:	0.953434 sec			Add Experiment 💌
IDA 0 min - 75 min	▼ Source and Gas Parar Ion source gas 1 Ion source gas 2	40 psi 40 psi	Curtain gas CAD gas	30 ‡ 7 ‡	Temperature	450 C	
	Experiment IDA Polarity TOF MS TOF start mass TOF stop mass	Positive V 350 Da 2000 Da	Spray voltage Declustering potential DP spread	5500 V 100 V 0 V	Collision energy CE spread	10 C V 0 V	
	IDA Criteria Peptide Maximum candidate ions Intensity threshold exceeds		Dynamic background Exclude former candid For 6 After 2	subtraction date ions sec ccurrences	Dynamic CE for MS/M Charge state tr	S 1 * 7 *	
	TOF MSMS Precursor ion TOF start mass TOF stop mass	830 \$ Da 50 \$ Da 2000 \$ Da	Declustering potential DP spread Accumulation time	100 ↓ V 0 ↓ V 0.05 ↓ sec	Collision energy CE spread	35 \$V 15 \$V	



B

Batch

In the 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.

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		Auto-	Calibrate Plate Layout	New	Open	✓ Save	♥ Print	Manage 🔹 👻	Submit	8
Untit	led									
	Sample Name	MS Method		LC Method		Rack code	Vial position	Data File		Â
1	Intact protein	intact protein analy:	sis MS	Intact_10	Dmin	1.5mL (105 vial)	1	Intact protein file		
2										
3										
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9		Batch - Automatic C	Calibration Editor				×			
10										
11		Provide ion reference and	calibrant delivery settings to	o be applied au	tomatically, at	the correct frequency dur	ing acquisition			
12										
13		Ion reference table	X500 ESI Positive Calibratio	on Solu 💙		Edit				
14		Calibrate every	APCI Negative Calibration S	Solution s	amples					
15		-	APCI Positive Calibration Sc	olution						_
10		Calibrant delivery	Beta Galactosidase Digests			CDS channel 1	♥			
19			Bovine Insulin							
19			CsI_ALILTLVS Peptide			or	Cancel			
20			ESI Positive Calibration Solu	ution		UN	Calicer			
21			Glu-fibrinopeptide B							
22			PPG Negative Calibration S	olution						
23			PPG Positive Calibration So	lution						
24			X500 ESI Negative Calibrati	ion Solution						
25			X500 ESI Positive Calibratio	n Solution						
26										

Batch - Automatic	Batch - Automatic Calibration Editor								
Provide ion reference and	ন্দ d calibrant delivery settings to be applied automatically, at the correct frequency during acquis	ition							
Ion reference table	X500 ESI Positive Calibration Solu 💙 Edit								
Calibrate every	3 samples								
Calibrant delivery	CDS CDS channel	•							
	OK								



Data Processing

Process biotherapeutic peptide mapping data in BioPharmaView[™] Software 2.0.

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

	Rituvimah	<u> </u>							E		
	Rituximab	4							Create	Open Sa	ve Save
	Assay Information	Sequence Fea	atures Inta	ct Protein Peptide Mapp	ing						
nformation 🔸	Protein Sequence										
Protein	Protein Type: Antibody	y 👻 🛛 Add Ch	hain U	Inmodified Protein MWs:							
terize Standard			N	Monoisotopic: 144195.31	89 Average: 144286.	27					
Batch											
Develo	Chain 1 Light Chain1										Delete C
Results	1-100 OTVI	SOSPATLSASPGE	KUTMTCRA	SSSVSYTHWFOOKPGS	SPEPWIYATSNIA	SGVPVRFSGSGSG	TSYSLTIS	RVEAFI	ATYYC	OOWTSNPP	TEGGG
e Mapping	101-200 TKLE	IKRTVAAPSVFIF	PPSDEQLE	SGTASVVCLLNNFYP	EAKVOWKVDNALO	SGNSQESVTEQDS	KDSTYSLS	STLTLS	KADYEK	HKVYACEV	THOGL
terize Standard	201-213 SSPV	TKSFNRGEC									
Batch	Chain 2 Heavy Chain	1									-
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	201-300 TYIC	NVNHKPSNTKVDK	KAEPKSCE	KTHTCPPCPAPELLGO	PSVFLFPPKPKDT	LMISRTPEVTCVV	VDVSHEDP	EVKENV	VYVDGVE	VHNAKTKP	REEQY
ueue	301-400 NSTY	RVVSVLTVLHODWI	LNGKEYKC	KVSNKALPAPIEKTI	KAKGOPREPOVYTI	LPPSRDELTKNOV	SLTCLVKG	FYPSDI	AVEWES	NGQPENNY	KTTPP
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	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS Chain 4 Light chain 2 AA Indee 1-100 QIVLS 101-200 TKLE1	DWYFRVWGAGTTV NVNHKPSNTKVDKI RVVSVLTVLHQDWI DGSFFLYSKLTVDI wes: SQSPAILSASPGEF IKRTVAAPSVFIFF	KAEPKSCI LNGKEYKO KSRWQQGN KVTMTCRA PPSDEQLK	NTHTCPPCPAPELLGC RVSNKALPAPIERTI IVFSCSVMHEALHNHYT SSSVSYIHWFQQKPGS SGTASVVCLLNNFYPF	PSVFLFPPKPKDT KAKGQPREPQVYT QKSLSLSPG SPRFWIYATSNLAS EAKVQWKVDNALQS	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS	VDVSHEDP SLTCLVKG TSYSLTIS KDSTYSLS	EVKFNW FYPSDI RVEAED STLTLS	NYVDGVE IAVEWES DAATYYC SKADYEK	OCWTSNPP HKVYACEV	REEQY KTTPP Delete C TFGGG THQGL
	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS Chain 4 Light chain 2 AA Inde 1-100 QIVLS 101-200 TKLE1 201-213 SSPVT	DMYFNWGAGTTU NVNHKESNTKVDKI RVVSVLTVLHQDMI DGSFFLYSKLTVDI X85: SQSPAILSASPGEK IKRTVAAPSVFIFF FKSFNRGEC	RAEPKSCI LNGKEYKC KSRWQQGN KVTMTCRA PPSDEQLK	NTHTCPPCPAPELLGC KVSNKALPAPIEKTI IVFSCSVMHEALHNHY SSSVSYIHWFQOKPGS SGTASVVCLLNNFYPF	PSVFLPPPKPKDTI KARGOPREPQVYTI VOKSLSLSPG SPRPWIYATSNLAS EAKVOWKVDNALQS	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS	VDVSHEDPI SLTCLVKG TSYSLTISI KDSTYSLS:	EVKFNW FYPSDI RVEAEI STLTLS	VYVDGVE LAVEWES DAATYYC SKADYEK	VHNAKTKPI NGQPENNYI QQWTSNPP HKVYACEV	REEQY KTTPP Delete C TFGGG THQGL
	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS Chain 4 Light chain 2 AA Inde: 1-100 QIVLS 101-200 TKLE1 201-213 SSPVT	DMYFNWGAGTTU NVNHKESNTKVDKI RVVSVLTVLHQDWI DGSFFLYSKLTVDI SQSPAILSASPGEP IKRTVAAPSVFIFF FKSFNRGEC	RAEPKSCI LNGKEYKC KSRWQQGN KVTMTCRA PPSDEQLK	KTHTCPPCPAPELLGC KVSNKALPAPIEKTI IVFSCSVMHEALHNHYT SSSVSYIHWFQCKPGS SGTASVVCLLNNFYPF Cysteine Mo	PSVFLPPPKPKDTI KARGOPREPQVYTI OKSLSLSPG SPRPWIYATSNLAS EAKVQWKVDNALQS difications Can Repla	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS ce Disulfide Bonds	VDVSHEDPI SLTCLVKGI TSYSLTISI KDSTYSLSS Disulfide B6	EVKFNV FYPSDI RVEAEL STLTLS	AATYYC KADYEK	VHNAKTRPI NGOPENNYI QQWTSNPP HKVYACEV Port	REEQY KTTPP Delete C TFGGG THQGL Export
	101-200 YYGG 201-300 TYIC 301-400 NSTY. 401-450 VLDS/ Chain 4 Light chain 2 AA Inde 1-100 QIVLS 101-200 TKLET 201-213 SSPVT Modifications Chains Type	DMYFNWGAGTTU NVNHRESNTKVDKI RVVSVLTVLHQDM DGSFFLYSKLTVDI XX05: SQSPAILSASPGEF IKRTVAAPSVFIFF FKSFNRGEC Name Pos	RAEPKSCI LNGKEYKC KSRWQQGN KVTMTCRA PPSDEQLK	KTHTCPPCPAPELLGC KVSNKALPAPIEKTI IVFSCSVMHEALHNHYT SSSVSYIHWFQOKPGS SGTASVVCLINNFYPP Cysteine Mc diffied Applies To	PSVFLPPPKPKDTI KARGQPREPQVYTI OKSLSLSPG SPRPWIYATSNLAS EAKVQWKVDNALQS difications Can Repla Workflow Usage	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS ce Disulfide Bonds Mass Shift	VDVSHEDPI SLTCLVKGI TSYSLTISI KDSTYSLSS Disulfide Bo From	EVKFNW FYPSDI RVEAED STLTLS Onds - (To Chain	PAATYYC RAVEWES DAATYYC SKADYEK (16) Ing From Custoine	VHNARTRPI NGOPENNYI OQOWTSNPP HKVYACEV To Costeine	REEQY KTTPP Delete C TFGGG THQGL Export
	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS/ Chain 4 Light chain 2 AA Inde 1-100 QIVLS 101-200 TKLET 201-213 SSPVT Modifications Chains Type 1 1-4 N-terminal	DAYENVWGAGTTU' NVNHRESNTKVDKI RVVSVLTVLHQDWI DGSFFLYSKLTVDI SGSPAILSASPGEF IKRTVAAPSVFIFF FKSFNRGEC Name Pos Gin->ovro-Glu	RAEPRSCI LNGKEYKC KSRWQQGN KVTMTCRA PPSDEQLK sition Mo AA	KTHTCPPCPAPELLGC KVSNKALPAPIEKTI IVFSCSVMHEALHNHYT SSSVSYIHWFOOKPGS SGTASVVCLINNFYPP Cysteine Mc dified Applies To O O	PSVFLPPPKPKDTI KARGQPREPQVYTI OKSLSLSPG SPRPWIYATSNLAS EAKVOWKVDNALQS difications Can Repla Workflow Usage Both	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS ce Disulfide Bonds Mass Shift -17.0265	VDVSHEDP) SLTCLVKGI TSYSLTISI KDSTYSLSS Disulfide Bo From Chain 1 1	EVKFNW FYPSDI RVEAEL STLTLS onds - (To Chain 1	PYVDGVE LAVEWES DAATYYC SKADYEK (16) Ing From Cysteine 23	VHNARTRP NGOPENNYI QQWTSNPP HKVYACEV To Cysteine 87	REEQY KTTPP Delete C TFGGG THQGL Export
	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS/ Chain 4 Light chain 2 AA Inde 1-100 QIVLS 101-200 TKLE1 201-213 SSPVT Modifications Chains Type 1 1-4 N-terminal 2 1-4 Internal	DAYENVWGAGTTU' NVNHRESNTKVDKI RVVSVLTVLHQDWI DGSFFLYSKLTVDI SGSPAILSASPGEF IKRTVAAPSVFIFF FKSFNRGEC Name Pos Gln->pyro-Glu Deamidated	RAEPRSCI LNGREYKC KSRWQQGN KVTMTCRA PPSDEQLK sition Mo AA	KTHTCPPCPAPELLGC KVSNKALPAPIEKTI IVFSCSVMHEALHNHYT SSSVSYIHWFQQKPGS SGTASVVCLLNNFYPP Cysteine Mc dified Applies To Q Q Q Q Q NA NQR	PSVFLPPPKPKDTI KARGOPREPGVYTI 'OKSLSLSPG SPRPWIYATSNLAS EAKVOWKVDNALQS difications Can Repla Workflow Usage Both Peptide Mapping	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS ce Disulfide Bonds Mass Shift -17.0265 0.9840	VDVSHEDP) SITCLVKG TSYSLTISE KDSTYSLS: Disulfide Bo From 1 1 1 2 1	EVKFNW FYPSDI STLTLS Conds - (To Chain 1 1	AVEWES AVEWES AATYYC KADYEK From Cysteine 23 133	OQWTSNPP HKVYACEV To Cysteine 87 193	REEQY KTTPP Delete C TFGGG THQGL Export
	101-200 YYGG 201-300 TYIC 301-400 NSTY 401-450 VLDS/ Chain 4 Light chain 2 AA Inde 1-100 QIVLS 101-200 TKLE1 201-213 SSPVT Modifications Chains Type 1 1-4 N-terminal 2 1-4 Internal 3 1-4 Internal	DAYENVWGAGTTU' NVNHRESNTRVDKI RVVSVLTVLHQDWI DGSFFLYSKLTVDI SGSPAILSASPGEF IKRTVAAPSVFIFF FKSFNRGEC Name Pos Gin->pyro-Glu Deamidated Oxidation	RAEPRSCI LNGREYKC KSRWQQGN KVTMTCRA PPSDEQLK sition Mo AA	NTHTCPPCPAPELLGC KVSNRALPAPIEKTI IVFSCSVMHEALHNHYT SSSVSYIHWFQQKPGS SGTASVVCLLNNFYPF Cysteine Mc dified Applies To Q Q Q Q Na NQR n/a MWHCDNYFKPR	PSVFLPPPKPKDTI KARGOPREPGVYTI 'OKSLSLSPG SPRPWIYATSNLAS EAKVOWKVDNALOS difications Can Repla- Workflow Usage Both Peptide Mapping Peptide Mapping	LMISRTPEVTCVV LPPSRDELTKNOV SGVPVRFSGSGSG SGNSQESVTEQDS ce Disulfide Bonds Mass Shift -17.0265 0.9840 15.949	VDVSHEDPI SLTCLVKGI TSYSLTISI KDSTYSLS: Disulfide Bc From Chain 1 1 2 1 3 1	EVKFNW FYPSDI RVEAEL STLTLS Onds - (To Chain 1 1 2	AVEWES AVEWES DAATYYC SKADYEK From Cysteine 23 133 213	VUHAAKTKP INGOPENNYI OQOWTSNPP HKVYACEV Oort 70 Cysteine 87 193 224	REEQY KTTPP Delete C TFGGG THOGL Export
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Navigate to the 'Peptide Mapping' tab complete processing parameters and to generate all peptide forms for matching.

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S	SSPVTKSF	NRGEC										
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N	STYRVVS	VLTVLH	QDWLNGKE	YKCKVSNKAI	PAPIEKT	ISKAKGOPREPOVYTL	PPSRDELT	KNQVSLTCI	VKGFYF	SDIAVE	WESNGOPE	NNYKTTPP
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Navigate to the 'Settings' icon and review your global 'Peptide Mapping Settings'

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Data extraction, including peptide matching can be performed in minutes, on either a single datafile, or on multiple samples using the batch processing function. Review your peptide mapping results in the BioPharmaView Software window. Full sequence coverage of matched peptides can be viewed by clicking 'View Sequence'. Peptide matches can be reviewed in the 'Peptide Results' window. For each selected peptide, corresponding TOF-MS raw spectrum (lower left) and high-resolution, annotated MS/MS spectrum (lower right) are shown for easy confirmation.



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