Applied Biosystems/MDS Analytical Technologies LC/MS Peptide/Protein Mass Standards Kit Protocol

This protocol includes the following sections:

1 Product description

Overview

The Applied Biosystems/MDS Analytical Technologies LC/MS Peptide/Protein Mass Standards Kit (Part Number 4368624) is designed for use with the following Applied Biosystems/MDS Analytical Technologies mass spectrometers:

- QSTAR® systems
- QSTAR® Pulsar systems
- QSTAR® XL systems
- QSTAR® Elite systems
- QTRAP® systems
- 3200 QTRAP® systems
- 4000 QTRAP® systems
- QTRAP® 5500 systems

The kit includes standards needed to quickly test these instruments to confirm they are functioning properly when performing electrospray analysis.

Applications

Prepare and use the standards in this kit to:

- **Evaluate MS mode** – The 6-Peptide Mixture contains peptides in the 900- to 3,700-Da mass range. During electrospray MS analysis, these peptides form multiply charged ions in the 300- to 1,300-m/z range. Analyze the peptides of this standard in MS mode and evaluate peak intensities to test sensitivity of the mass spectrometer when performing MS, direct-syringe-infusion analyses using an IonSpray™, TurboIonSpray®, or TurboV™ source.
- **Evaluate MS/MS mode** – The 6-Peptide Mixture contains Glu[1]-Fibrinopeptide B, which contains fragment ions in the 100- to 1,500-m/z range. Perform MS/MS analysis on this peptide and evaluate peak intensities of peptide fragment ions to test sensitivity of the mass spectrometer when performing MS/MS, direct-syringe-infusion analyses using an IonSpray, TurboIonSpray, or TurboV source.
- **Evaluate MS and MS/MS mode with One Pure Peptide** – There is a separate vial containing purified Glu[1]-Fibrinopeptide B. This peptide standard is used to perform the NanoSpray® Source Installation and Site Acceptance tests. These tests are performed by an Applied Biosystems Service Engineer during installation.

- **Evaluate LC/MS/MS mode** – The tryptic-digested Beta-Galactosidase provided is a complex mixture of peptides that typically requires LC separation before MS analysis. Perform an LC/MS/MS analysis on this standard and evaluate the extracted ion chromatograms of select peptides to test separation efficiency of the LC system and sensitivity of the mass spectrometer when performing LC/MS/MS analyses using a NanoSpray® source.

2 Materials

Materials in the kit

The LC/MS Peptide/Protein Mass Standards Kit includes the following:

- **Glu[1]-Fibrinopeptide B - one vial (0.1 mg, Sigma catalog # F-3261)**
- **6-Peptide Mixture** – three vials (61.2 µg/vial)
- **Beta-Galactosidase, Digested** – two vials (72.8 µg/vial)
- **Standard Diluent with 0.1% Formic Acid** – 30% acetonitrile in 0.1% formic acid, 4 vials (1 mL/vial)
- **Empty vials with caps** – Ten 0.5-mL vials with caps

Table 1 Standard mixtures – components, sequences, and molecular weights

Standard	Component	Peptide sequence or accession number	Monoisotopic molecular weight (Da)
6-Peptide Mixture	Bradykinin (2–9 clip)	PPGFSPFR	903.5
	Angiotensin I, human	DRVYIHPFHL	1,295.7
	Glu[1]-Fibrinopeptide B	EGVNDNEEGFFSAR	1,569.7
	ACTH (1–17 clip)	SYSMEHFRWGKPVGKKR	2,092.1
	ACTH (18–39 clip)	RPVKVYPNGAEDESAEAFPLEF	2,464.2
	ACTH (7–38 clip)	FRWGKPVGKKRRPVKVYPNGAEDESAEAFPLE	3,656.9
Beta-Galactosidase, Digested	Beta-Galactosidase, *Escherichia coli*, digested with bovine trypsin and lyophilized	gi|114939	116,410
Glu-Fib peptide	Glu[1]-Fibrinopeptide B	EGVNDNEEGFFSAR	1569.7

Note: For mass assignments of the components, see Tables 3, 4, and 5 on pages 20 and 21.

Table 2 Standard mixtures – components and concentrations

Standard	Component	µg per vial	Concentration in stock solution (pmol/µL)	Concentration in final solution (fmol/µL)		
				QSTAR® Systems	QTRAP® / 3200 QTRAP® systems	QTRAP® 5500/4000 QTRAP® systems
6-Peptide Mixture	Bradykinin (2–9 clip)	2.3	10.2	101.8	203.7	50.9
	Angiotensin I, human	6.5	20.1	200.7	401.3	100.3
	Glu[1]-Fibrinopeptide B	5.1	13.0	130.0	259.9	65.0
	ACTH (1–17 clip)	10.5	20.1	200.7	401.5	100.4
	ACTH (18–39 clip)	9.3	15.1	151.0	301.9	75.5
	ACTH (7–38 clip)	27.5	30.1	300.8	601.6	150.4
Beta-Galactosidase, Digested	Beta-Galactosidase, *Escherichia coli*, digested with bovine trypsin and lyophilized	72.8	1.0	100.0	100.0	100.0
Glu-Fib peptide	Glu[1]-Fibrinopeptide B	100	50	150	250	50

Materials you provide

- Pipettors
- Pipette tips
- Aqueous LC mobile phase

3 Prepare reagents

This section includes:

- Prepare 6-Peptide Mixture
- Prepare Beta-Galactosidase
- Prepare the [Glu[1]]-Fibrinopeptide B

Preparation guidelines

Recommended pipette volume ranges

For best results, use a pipette with a volume range appropriate for the volumes you pipette. When pipetting 0.5- to 1-µL volumes, use a pipette with a volume range of 0.5 µL to 2.0 µL or 0.5 µL to 2.5 µL.

IMPORTANT! Do not use a 1-µL to 10-µL pipette when pipetting volumes ≤ 1 µL. Volumes at the low end of the pipette volume range can be inaccurate. If using a 1-µL to 10-µL pipette is unavoidable, double or triple the volumes in *all* steps to ensure an accurate dilution.

Solution stability

Solution	Stability information
Stock Solution	• Prepare stock solution immediately before use. • Aliquot stock solution in 50-µL volumes. Freeze unused aliquots at ≤ – 20 °C for future use. • Do not repeatedly freeze and thaw. Doing so can degrade the standard.
Final Solutions	• Prepare final solutions immediately before use. • Refrigerate at 4 °C when not in use for up to 3 days. • If you do not use a final solution within 3 days, discard. Do not freeze final solutions.

Prepare 6-Peptide Mixture

1. Make a Stock Solution by adding 250.0 µL of Standard Diluent with 0.1% Formic Acid to the 6-Peptide Mixture vial.
2. Vortex the vial for at least 30 seconds.
3. Centrifuge the vial for 5 seconds.
4. Repeat steps 2 through 3.
5. Aliquot the Stock Solution in 50-µL volumes. Freeze unused aliquots for future use.
6. Make a Final Solution by using the Standard Diluent with 0.1% Formic Acid to dilute the Stock Solution to the following volumes:

Instrument	Dilute this volume of stock solution	To a final volume of...
QSTAR®, QSTAR® Pulsar, and QSTAR XL systems	2.0 µL	200.0 µL
QTRAP® and 3200 Q TRAP® systems	4.0 µL	200.0 µL
4000 QTRAP®/QTRAP® 5500 systems	1.0 µL	200.0 µL

7. Refrigerate Final Solutions at 4 °C for up to 3 days.

Prepare Beta-Galactosidase

1. Make a Stock Solution by adding 625.0 µL of Standard Diluent with 0.1% Formic Acid to the Beta-Galactosidase vial.
2. Vortex the vial for at least 30 seconds.
3. Centrifuge the vial for 5 seconds.
4. Repeat steps 2 through 3.
5. Aliquot the Stock Solution in 50-µL volumes. Freeze unused aliquots for future use.
6. Make a Final Solution by using your aqueous LC mobile phase to dilute 10.0 µL of the Stock Solution to 100.0 µL.
7. Refrigerate Final Solutions at 4 °C for up to 3 days.

   IMPORTANT! If you want to make a more concentrated Final Solution, make sure the total organic content of the final solution is < 5%. An organic content above 5% prevents the analyte from properly adhering to the column.

Prepare the [Glu^1]-Fibrinopeptide B

1. Make a stock solution by adding 600 µL × 2 (total of 1200 µL) of Standard Diluent (0.1% Formic Acid, 30% ACN) to 0.1 mg [Glu^1]-Fibrinopeptide B amber glass vial.
2. Shake with the cover on, then vortex the vial for at least 2 minutes.

   IMPORTANT! The peptide must be fully dissolved before proceeding.
3. Cover the vial tightly, then flick the vial so that all solution goes to bottom of the vial. This preparation will create a 50 pmol µL of Stock Solution.
4. Aliquot the Stock Solution in 50-µL volumes. Freeze unused aliquots for future use.
5. Make a Final Solution by using the Standard Diluent with 0.1% Formic Acid to dilute the Stock Solution to the following volumes:

Instrument	Dilute the volume of stock solution	To a final volume of...
QSTAR®, QSTAR® Pulsar, and QSTAR® XL Systems	3 µL	1000 µL
QTRAP® and 3200 QTRAP® Systems	5 µL	1000 µL
4000 QTRAP® and QTRAP® 5500 Systems	1 µL	1000 µL

6. Vortex the vial for at least 30 seconds. Centrifuge the vial in a microcentrifuge for 10 seconds to bring the solution to the bottom of the tube. Repeat vortexing and centrifuging the vial to ensure dissolution.

   IMPORTANT! Before proceeding with the analysis, put the remainder of the stock solution and [Glu^1]-Fibrinopeptide B at – 20 °C.

4 Analyze standards

Standards to analyze

Analyze the standard needed for your application. For representative spectra and mass assignments for standards, see Section 5, "Spectra." and Section 6, "Masses."

Application	Standard required
Test sensitivity of mass spectrometer when performing MS, direct-syringe-infusion analyses using an IonSpray™, TurboIonSpray®, or TurboV™ source.	6-Peptide Mixture
Test sensitivity of mass spectrometer when performing MS/MS, direct-syringe-infusion analyses using an IonSpray, TurboIonSpray, or TurboV source.	6-Peptide Mixture
Test separation efficiency of the LC system and sensitivity of the mass spectrometer when performing LC/MS/MS analyses using a NanoSpray® source.	Beta-Galactosidase, Digested

Analyze standards using QSTAR®, QSTAR® Pulsar, QSTAR® XL, and QSTAR® Elite systems

TOF MS method for QSTAR®, QSTAR® Pulsar, QSTAR® XL, and QSTAR® Elite systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Ion Source Gas 1	20.0
	Curtain Gas	20.0
	IonSpray Voltage	5500.0
Compound	Declustering Potential	50
MS	Scan type	TOF MS
	Polarity	Positive
	TOF Masses (amu)	Min: 400 Max: 1800
	Cycles	30
Advanced MS	MCA	Select checkbox
	Q1 Transmission Window	Click **Suggest** to set values
	TOF Extraction Parameters	Click **Suggest** to set values

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. At the top of the window, select **Syringe Pump Method** from the drop-down list.
4. In the Syringe Pump Properties tab, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
5. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

Product ion method for QSTAR®, QSTAR® Pulsar, QSTAR® XL, and QSTAR® Elite systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Ion Source Gas 1	20.0
	Curtain Gas	20.0
	IonSpray Voltage	5500.0
Compound	Declustering Potential	50
	Collision Gas	5.0
	Collision Energy	40.0
Resolution	Q1 Resolution	Low Resolution (Offset Drop = 0.1)
MS	Scan type	Product Ion
	Product Of	785.8
	Polarity	Positive
	TOF Masses (amu)	Min: 100 Max: 1500
	Enhance All	Select checkbox
	Cycles	30
Advanced MS	MCA	Select checkbox
	Q1 Transmission Window	Click **Suggest** to set values
	TOF Extraction Parameters	Click **Suggest** to set values

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. At the top of the window, select **Syringe Pump Method** from the drop-down list.
4. In the Syringe Pump Properties tab, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
5. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

LC/MS/MS method for QSTAR®, QSTAR® Pulsar, QSTAR® XL, and QSTAR® Elite systems

Refer to the LC/MS and IDA tutorials that came with your system. For information on locating these tutorials, contact Technical Support using the information on the back page.

Analyze standards using QTRAP® and 3200 QTRAP® systems

EMS (enhanced MS) method for QTRAP® and 3200 QTRAP® systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	15.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	15.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	70.0
	Collision Energy	10.0
MS	Scan type	Enhanced MS (EMS)
	Polarity	Positive
	MCA	Select checkbox
	Start (amu) / Stop (amu)	400.000 / 1200.000
	Optimize Masses	Click button
	Number of scans to sum	2
	Cycles	10
Advanced MS	Scan rate (amu/s)	4000
	Fixed LIT fill time (ms)	20
	Q0 Trapping	Deselect checkbox

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. At the top of the window, select **Syringe Pump Method** from the drop-down list.
4. In the Syringe Pump Properties tab, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
5. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

EPI (enhanced product ion) method for QTRAP® and 3200 QTRAP® systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	15.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	15.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	70.0
	Collision Energy	38.0
Resolution	Q1 Resolution	Low (Offset Drop = 0.1)
MS	Scan type	Enhanced Product Ion (EPI)
	Polarity	Positive
	Product Of (amu)	785.800
	MCA	Select checkbox
	Start (amu) / Stop (amu)	100.000 / 280.000 275.000 / 1500.000
	Optimize Masses	Click button
	Number of scans to sum	2
	Cycles	10
Advanced MS	Scan rate (amu/s)	4000
	Q0 Trapping	Select checkbox
	Fixed LIT fill time (ms)	10

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. At the top of the window, select **Syringe Pump Method** from the drop-down list.
4. In the Syringe Pump Properties tab, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
5. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

LC/MS/MS method for QTRAP® and 3200 QTRAP® systems

Refer to the LC/MS and IDA tutorials that came with your system. For information on locating these tutorials, contact Technical Support using the information on the back page.

Analyze Standards Using 4000 QTRAP® systems

EMS (enhanced MS) method for 4000 QTRAP® systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	15.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	15.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	70.0
	Collision Energy	10.0
MS	Scan type	Enhanced MS (EMS)
	Polarity	Positive
	MCA	Select checkbox
	Start (amu) / Stop (amu)	400.000 / 1200.000
	Optimize Masses	Click button
	Number of scans to sum	2
	Cycles	10
Advanced MS	Scan rate (amu/s)	4000
	Fixed LIT fill time (ms)	5
	Q0 Trapping	Deselect checkbox

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. Using the external syringe pump, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
4. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

EPI (enhanced product ion) method for 4000 QTRAP® systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	15.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	15.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	70.0
	Collision Energy	38.0
Resolution	Q1 Resolution	Low (Offset Drop = 0.1)
MS	Scan type	Enhanced Product Ion (EPI)
	Polarity	Positive
	Product Of (amu)	785.600
	MCA	Select checkbox
	Start (amu) / Stop (amu)	100.000 / 280.000 275.000 / 1500.000
	Optimize Masses	Click button
	Number of scans to sum	2
	Cycles	10
Advanced MS	Scan rate (amu/s)	4000
	Q0 Trapping	Select checkbox
	Fixed LIT fill time (ms)	10

3. Using the external syringe pump, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
4. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

LC/MS/MS method for 4000 QTRAP® Systems

Refer to the LC/MS and IDA tutorials that came with your system. For information on locating these tutorials, contact Technical Support using the information on the back page.

Analyze Standards Using QTRAP® 5500 systems

EMS (enhanced MS) method for QTRAP® 5500 systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	20.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	20.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	100.0
	Collision Energy	10.0
MS	Scan type	Enhanced MS (EMS)
	Polarity	Positive
	MCA	Select checkbox
	Start (amu) / Stop (amu)	400.000 / 1000.000
	Optimize Masses	Click button
	Number of scans to sum	1
	Cycles	10
Advanced MS	Scan rate (Da/s)	10,000
	Fixed LIT fill time (ms)	0.5 ms
	Q0 Trapping	Deselect checkbox

Note: Leave all other parameters set to values optimized by the Applied Biosystems representative.

3. Using the external syringe pump, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
4. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

EPI (enhanced product Ion) method for QTRAP® 5500 systems

1. In the Analyst® Software, select **Manual Tuning**.
2. Select the following tabs, then set the indicated parameters:

Tab	Parameter	Setting
Source/Gas	Curtain Gas	20.0
	Collision Gas	High
	IonSpray Voltage	5500.0
	Temperature	0.0
	Ion Source Gas 1	20.0
	Ion Source Gas 2	0.0
	Interface Heater	Click On
Compound	Declustering Potential	100.0
	Collision Energy	47.0
Resolution	Q1 Resolution	Unit
MS	Scan type	Enhanced Product Ion (EPI)
	Polarity	Positive
	Product Of (amu)	785.9
	MCA	Select checkbox
	Start (amu) / Stop (amu)	100.000 / 280.000 275.000 / 1000.000
	Optimize Masses	Click button
	Number of scans to sum	1
	Cycles	10
Advanced MS	Scan rate (Da/s)	20,000
	Q0 Trapping	Off
	Fixed LIT fill time (ms)	10

3. Using the external syringe pump, set the **Syringe Diameter** appropriate for the syringe you are using, then set the Flow Rate to **10 µL/minute**.
4. At the top of the Analyst Software window, click **Start Syringe Pump**, then click **Start**.

LC/MS/MS method for QTRAP® 5500 systems

Refer to the LC/MS and IDA tutorials that came with your system. For information on locating these tutorials, contact Technical Support using the information on the back page.

5 Spectra

This section includes:

IMPORTANT! Masses are included in the spectra for peak identification only. Exact peak masses are listed in Tables 3, 4, and 5.

IMPORTANT! The following are representative spectra and should not be used as performance specifications.

Spectra for QSTAR®, QSTAR® Pulsar, QSTAR® XL, and QSTAR® Elite systems

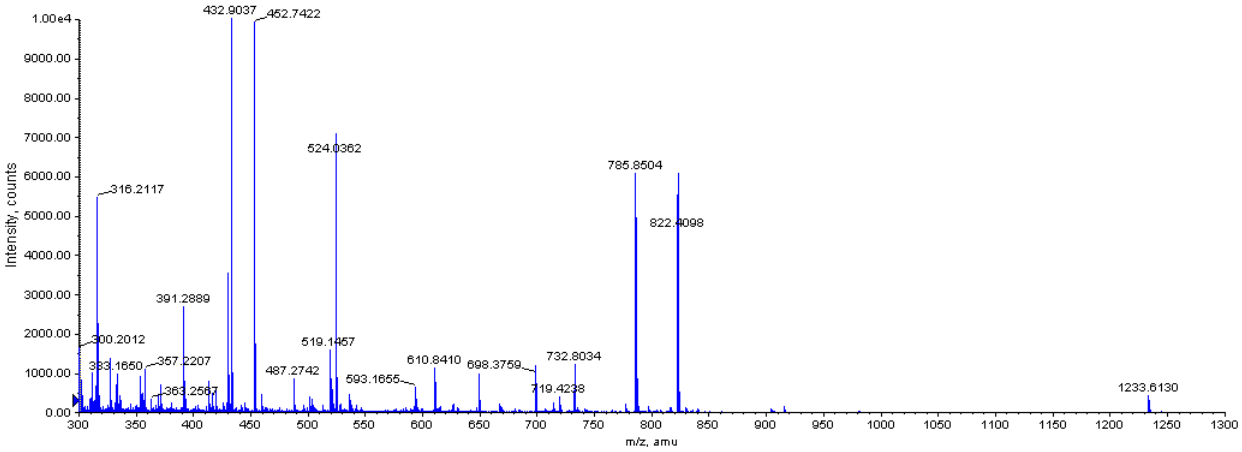

Figure 1 TOF MS spectrum of 6-Peptide Mixture

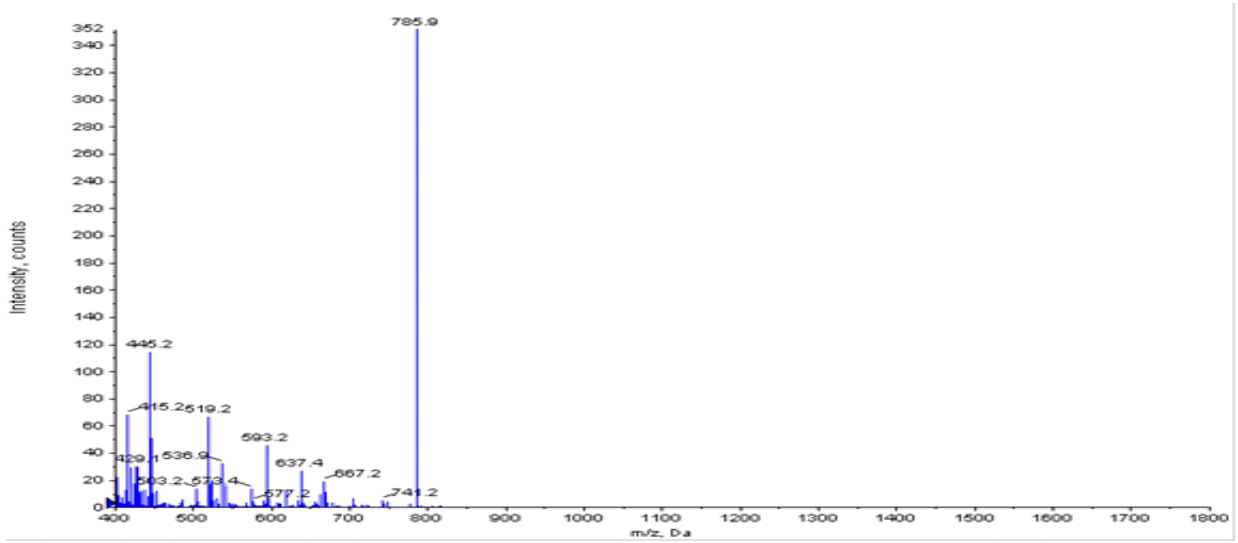

Figure 2 TOF MS spectrum of spectrum of Glu1-Fibrinopeptide B

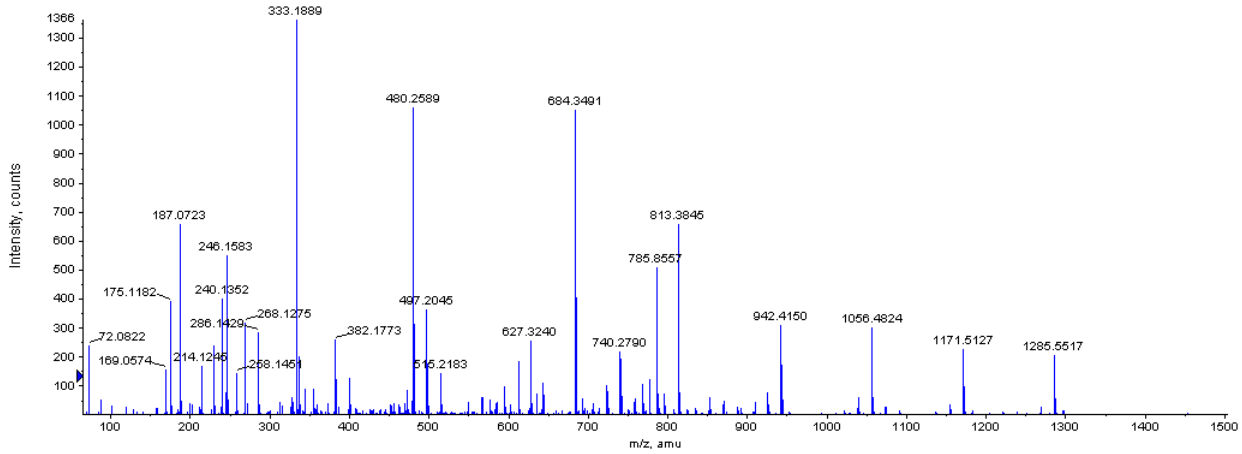

Figure 3 Product Ion Spectrum of Glu1-Fibrinopeptide B

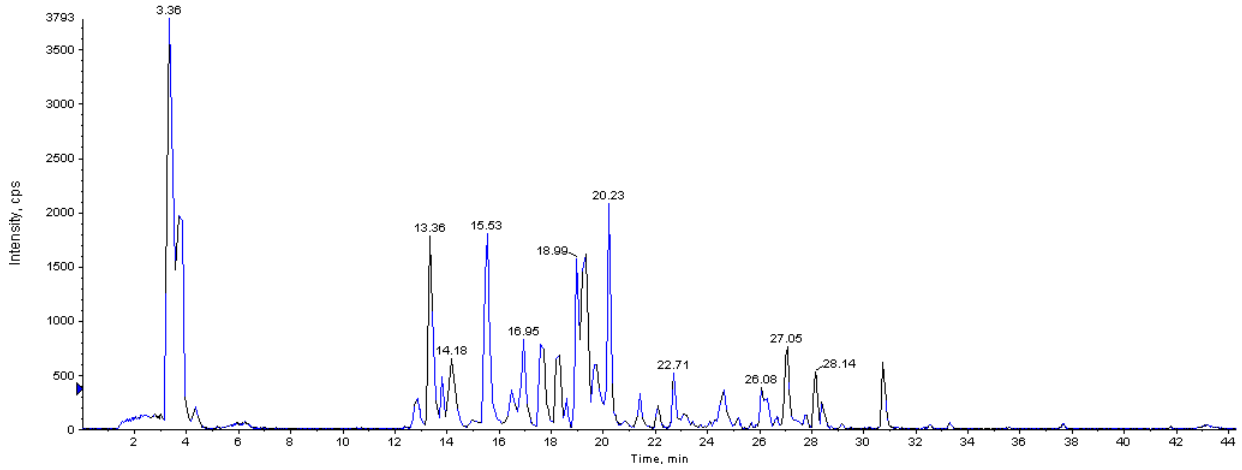

Figure 4 LC/MS/MS BPC (base peak chromatogram) of Beta-Galactosidase tryptic digest

Spectra for QTRAP® and 3200 QTRAP® systems

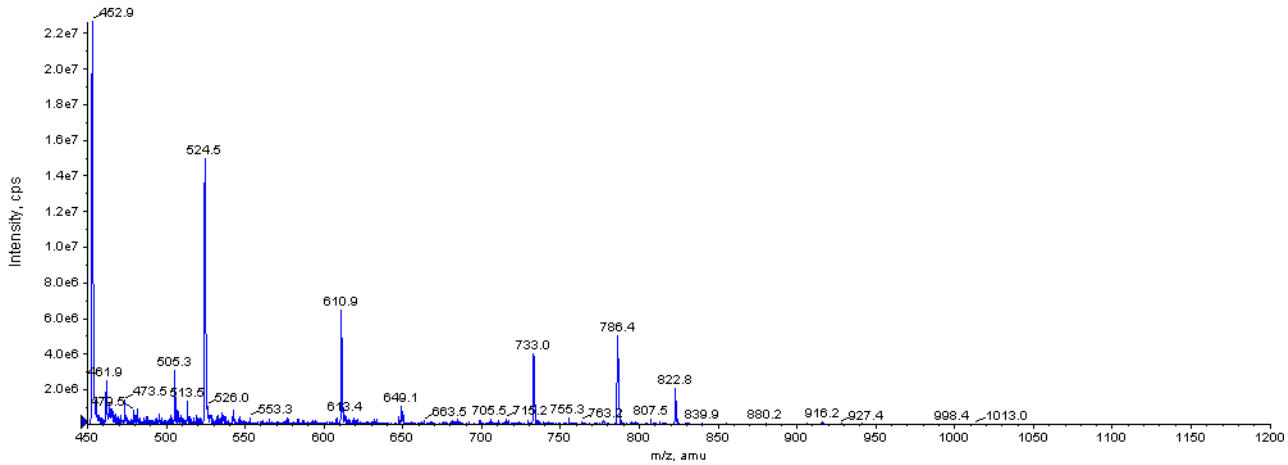

Figure 5 EMS (Enhanced MS) spectrum of 6-Peptide Mixture

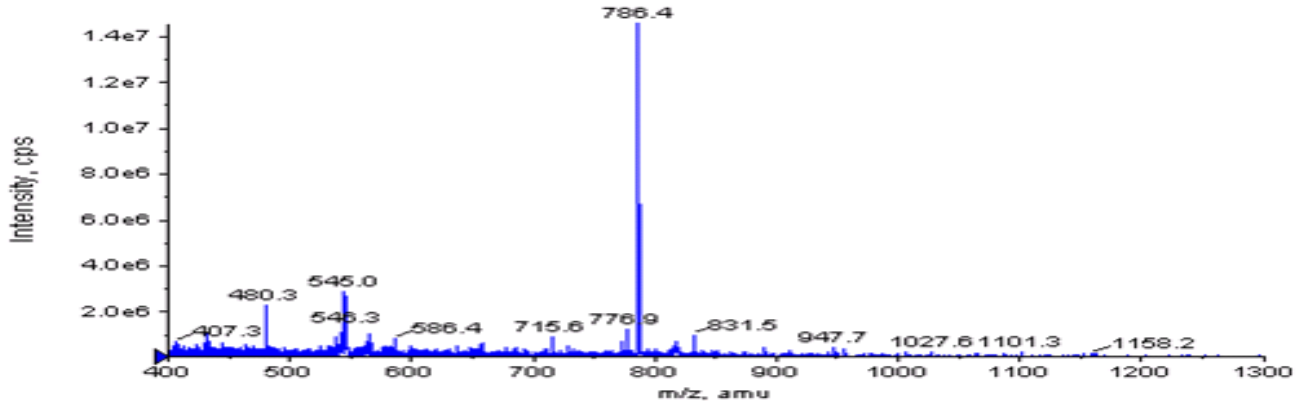

Figure 6 EMS (Enhanced MS) spectrum of Glu1-Fibrinopeptide B

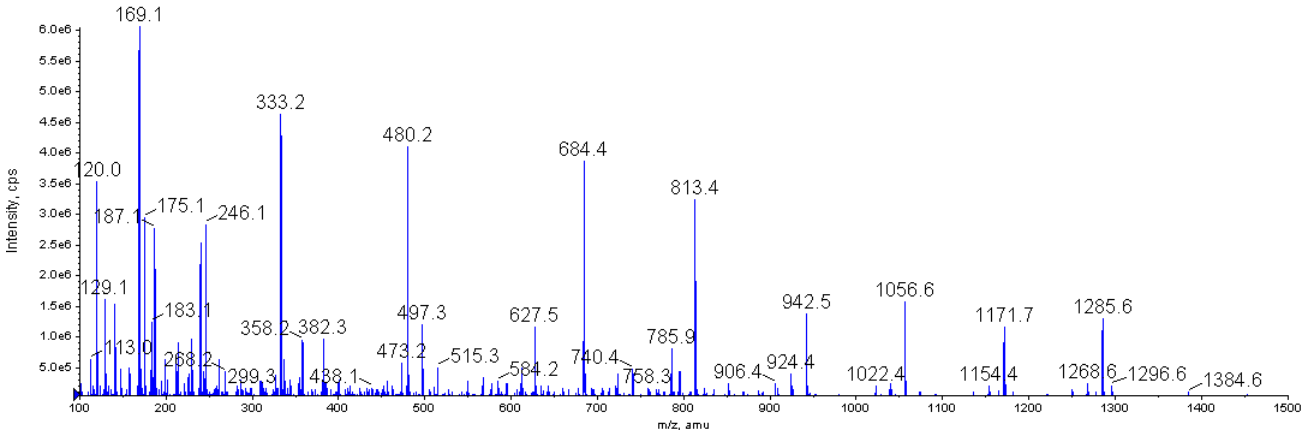

Figure 7 EPI (Enhanced Product Ion) spectrum of Glu1-Fibrinopeptide B

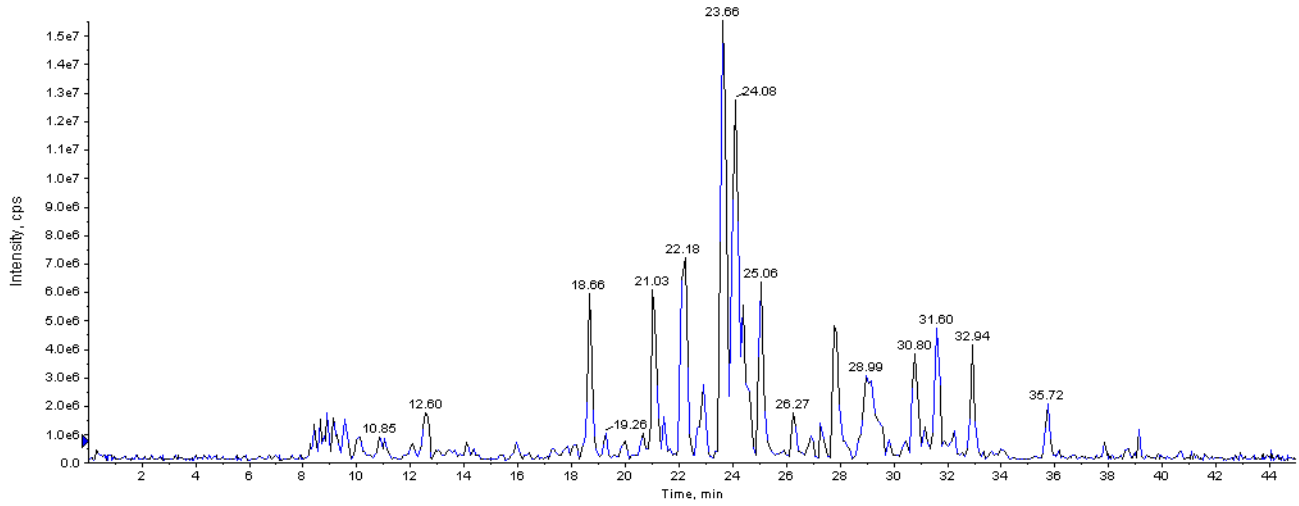

Figure 8 LC/MS/MS BPC (base peak chromatogram) of Beta-Galactosidase tryptic digest

Spectra for 4000 QTRAP® systems

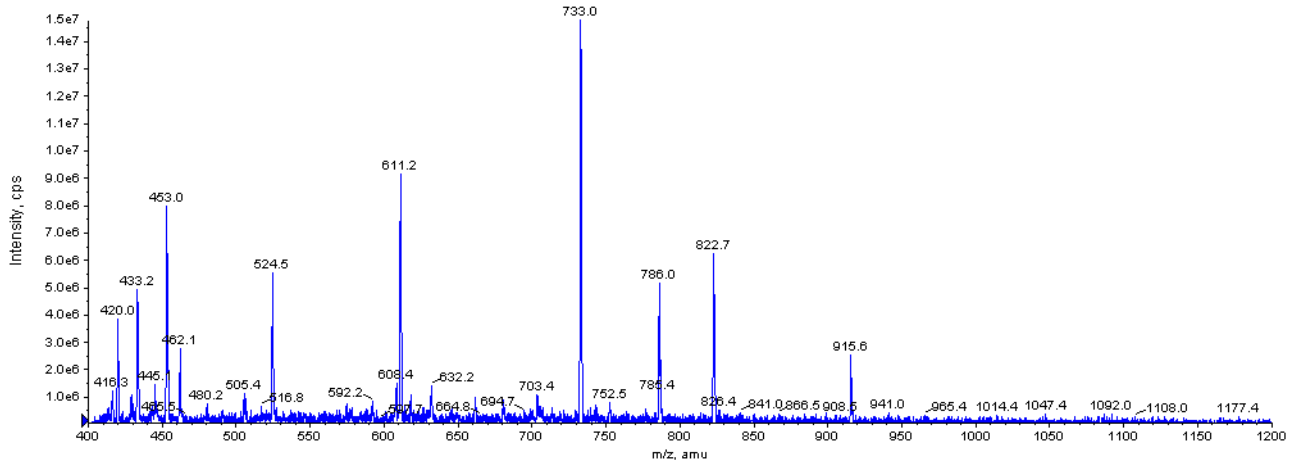

Figure 9 EMS (Enhanced MS) spectrum of 6-Peptide Mixture

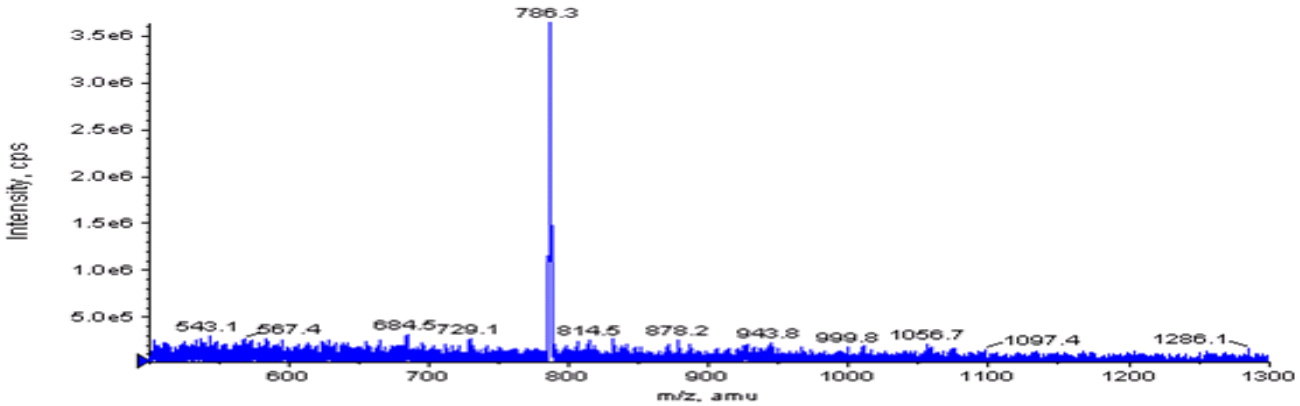

Figure 10 EMS (Enhanced MS) spectrum of Glu1-Fibrinopeptide B

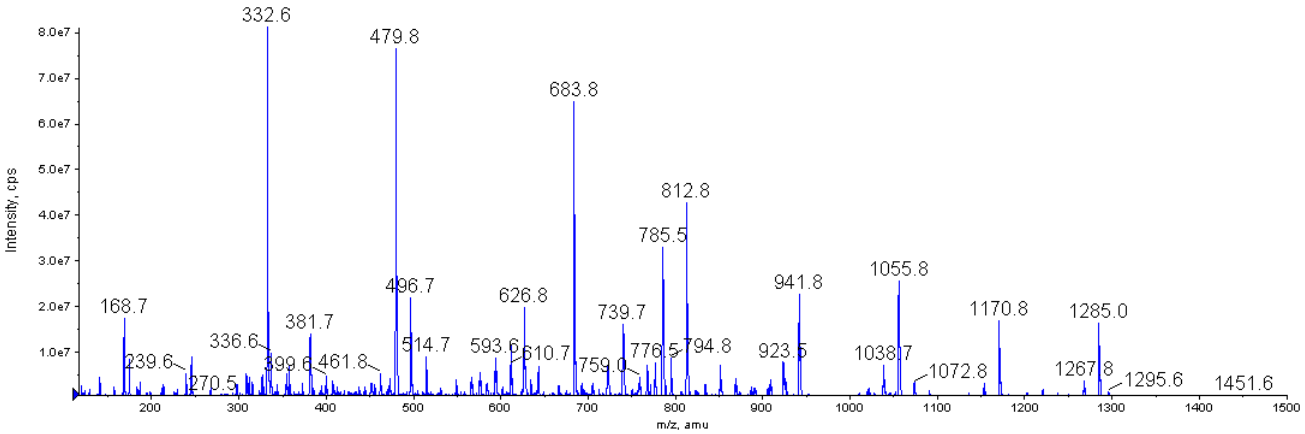

Figure 11 EPI (Enhanced Product Ion) spectrum of Glu1-Fibrinopeptide B

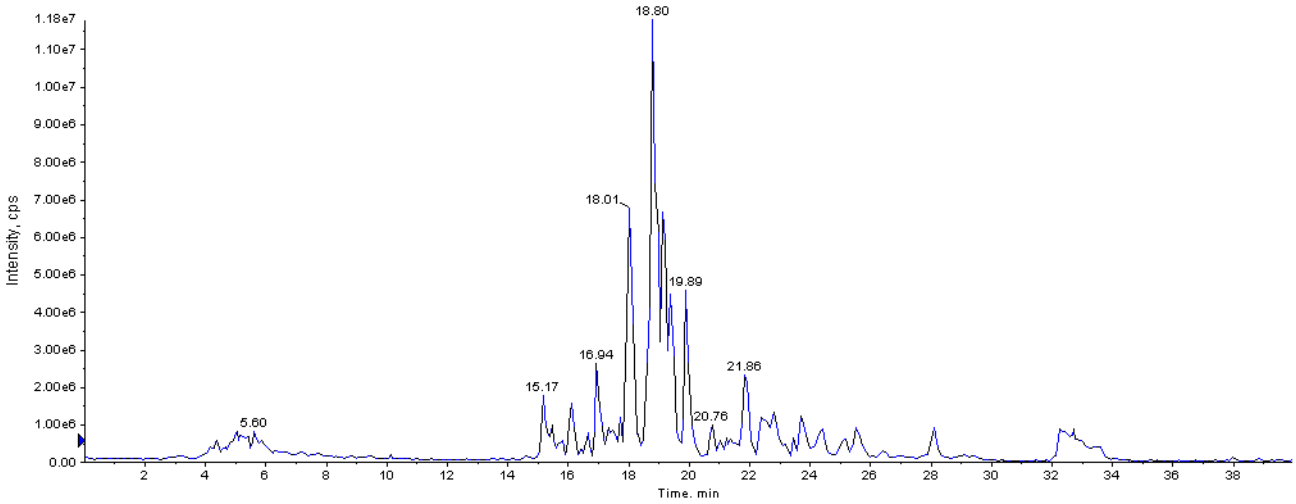

Figure 12 LC/MS/MS BPC (base peak chromatogram) of Beta-Galactosidase tryptic digest

Spectra for QTRAP® 5500 systems

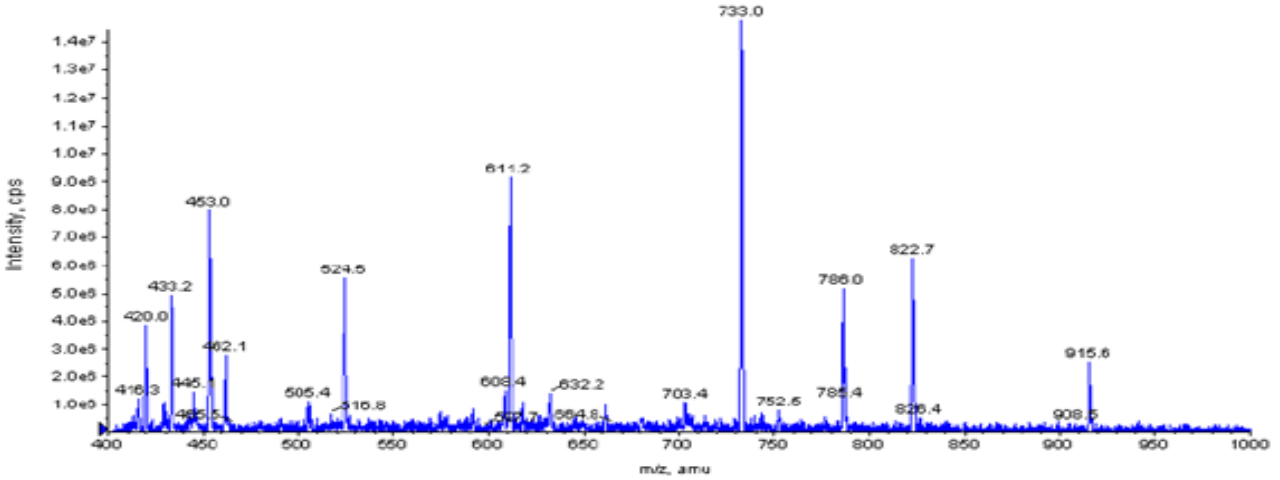

Figure 13 EMS (Enhanced MS) spectrum of 6-Peptide Mixture

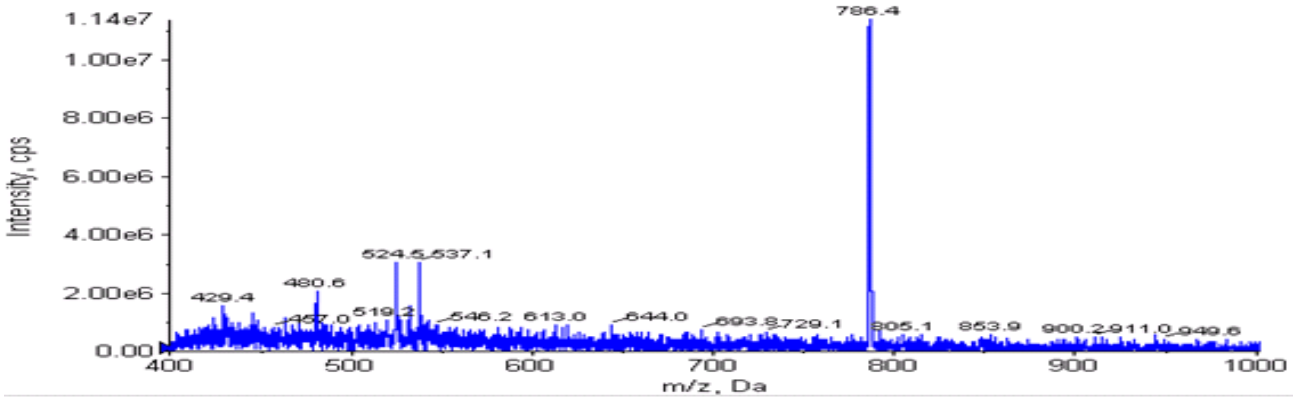

Figure 14 EMS (Enhanced MS) spectrum of Glu1-Fibrinopeptide B

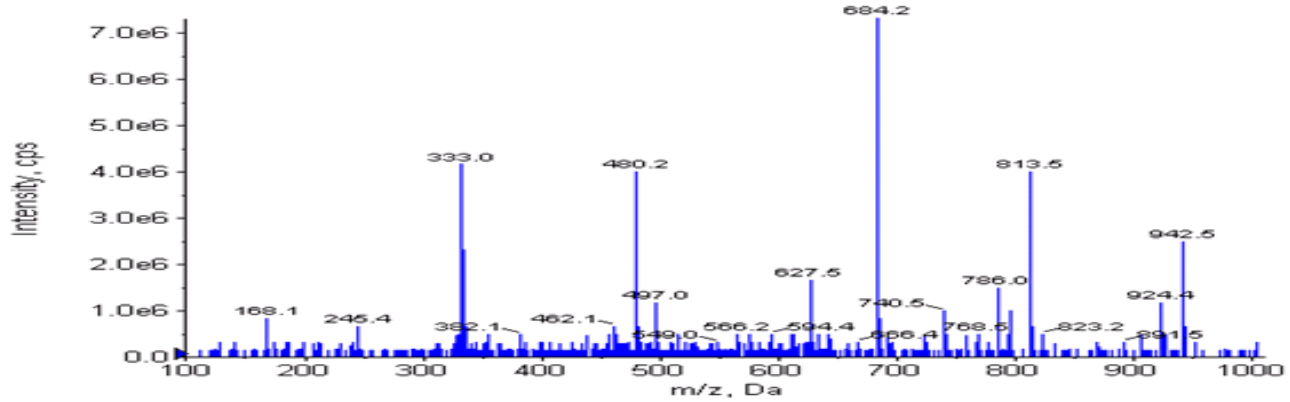

Figure 15 EPI (Enhanced Product Ion) spectrum of Glu1-Fibrinopeptide B

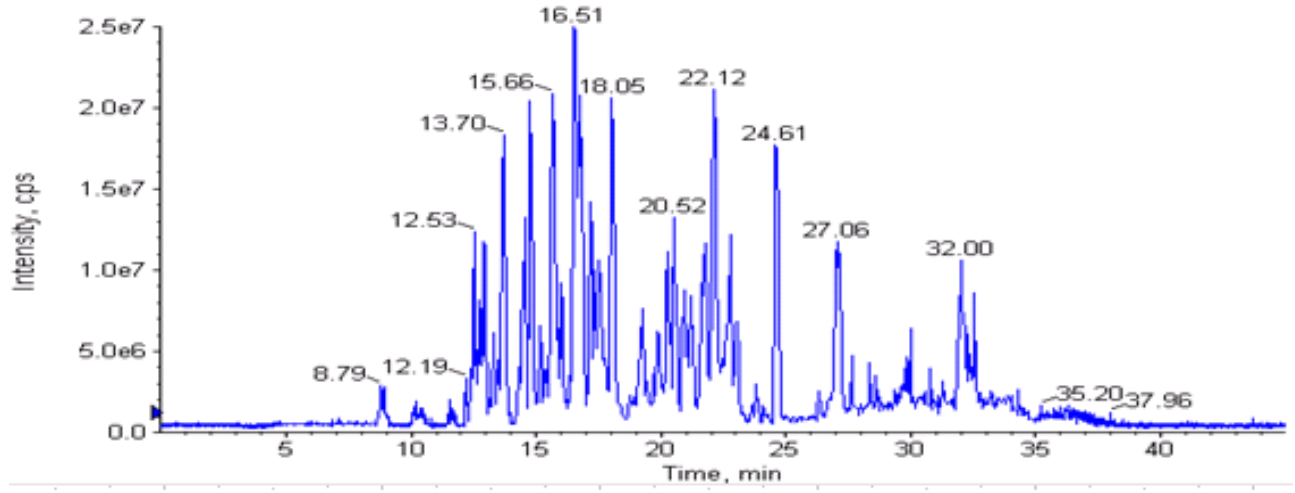

Figure 16 LC/MS/MS BPC (base peak chromatogram) of Beta-Galactosidase tryptic digest

6 Masses

Use the masses listed in Tables 3, 4, and 5 for calibration.

Table 3 Mass assignments for 6-Peptide Mixture

Peptide	Monoisotopic molecular weight (Da)	$(M+nH)n^+$ monoisotopic m/z for charge of …					
		+1	+2	+3	+4	+5	+6
Bradykinin (2–9 clip)	903.4603	904.4676‡	452.7374‡	302.1607	—	—	—
Angiotensin I, human	1,295.6775	1296.6848	648.8460‡	432.8998‡	324.9266	—	—
Glu[1]-Fibrinopeptide B	1,569.6696	1570.6768	785.8421‡	524.2305‡	393.4247	—	—
ACTH (1–17 clip)	2,092.0789	—	1,047.0467	698.3669‡	524.0270‡	419.4231‡	349.6871
ACTH (18–39 clip)	2,464.1911	—	1,233.1028‡	822.4043‡	617.0550	493.8455	—
ACTH (7–38 clip)	3,656.9216	—	—	1219.9811	915.2377‡	732.3916‡	610.4942‡

‡. Indicates more commonly observed charge states.

Table 4 lists monoisotopic m/z values for the theoretical cleavages of Glu1-Fibrinopeptide B, as calculated for the positive ion mode.

Table 4 Theoretical fragment ions of Glu1-Fibrinopeptide B

b Ions		y Ions	
(m/z)	Fragment	(m/z)	Fragment
—	—	1570.6768	EGVNDNEEGFFSAR
130.0499	E	1441.6342	GVNDNEEGFFSAR
187.0713	EG	1384.6128	VNDNEEGFFSAR
286.1397	EGV	1285.5444	NDNEEGFFSAR
400.1827	EGVN	1171.5014	DNEEGFFSAR
515.2096	EGVND	1056.4745	NEEGFFSAR
629.2525	EGVNDN	942.4316	EEGFFSAR
758.2951	EGVNDNE	813.3890	EGFFSAR
887.3377	EGVNDNEE	684.3464	GFFSAR
944.3592	EGVNDNEEG	627.3249	FFSAR
1091.4276	EGVNDNEEGF	480.2565	FSAR
1238.4960	EGVNDNEEGFF	333.1881	SAR
1325.5281	EGVNDNEEGFFS	246.1561	AR
1396.5652	EGVNDNEEGFFSA	175.1190	R
1552.6663	EGVNDNEEGFFSAR	—	—

Table 5 Mass assignments for ten abundant peptides in Beta-Galactosidase digest

Fragment number	Peptide fragment	Monoisotopic molecular weight (Da)	m/z	Charge state
T64	IDPNAWVER	1,098.5458	550.2802	2
T41	VDEDQPFPAVPK	1,340.6612	671.3379	2
T63	APLDNDIGVSEATR	1,456.7158	729.3652	2
T14	WSDGSYLEDQDMWR	1,786.7257	894.3701	2
T77	GDFQFNISR	1,082.5145	542.2654	2
T24	LWSAEIPNLYR	1,360.7139	681.3642	2
T3	DWENPGVTQLNR	1,427.6793	714.8469	2
T10	WVGYGQDSR	1,066.4832	534.2489	2
T48	YDENGNPWSAYGGDFGDTPNDR	2,445.9734	816.3317	3
T72	VNWLGLGPQENYPDR	1,756.8533	879.4339	2

7 Store the kit

For best results, prepare the standards immediately before use. For more information, see “Solution stability” on page 4.

Store the LC/MS Peptide/Protein Mass Standards Kit and components of the kit under the following conditions. Avoid prolonged exposure to light.

Kit component	Storage temperature	Stability
Unopened kit	– 20 °C	1 year from date of shipment
Stock solution	– 20 °C	6 months If maintained at – 20 °C continually. (Freezing and thawing repeatedly can cause the standard solution to degrade.)
Final solutions	4 °C	3 days

8 Accessories, spare parts, and ordering information

Item	Quantity	Part number
Applied Biosystems/MDS Analytical Technologies LC/MS Peptide/Protein Mass Standards Kit	1 kit	4368624

9 Safety

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

IMPORTANT! Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See "About MSDSs" below.
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to https://docs.appliedbiosystems.com/msdssearch.html
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click Search.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **E-mail** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical waste hazard

WARNING **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical waste guidelines

To minimize the hazards of chemical waste:

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

10 Technical support

Applied Biosystems is committed to meeting the needs of your research through enabling technologies like the Applied Biosystems/MDS Analytical Technologies LC/MS Peptide/Protein Mass Standards Kit. Our dedicated support staff is available to answer questions about using this product to the fullest extent possible.

Applied Biosystems offers a suite of LC/MS systems to meet your proteomics applications needs. Please contact your Applied Biosystems representative for technical and ordering information.

Applied Biosystems publishes a continuing series of Application Notes. For a publications list, or for further details or answers to questions related to other products, contact Applied Biosystems using the information on the back page of this document.

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