



Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments

Protocol

1 Product Description

The Mass Standards Kit includes reagents needed to test instrument function, optimize instrument parameters, and calibrate the mass scale using known peptide standards.

Applications

Using the mixtures of known peptides and proteins in the Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments, you can monitor results in a mass range of 900 to 3,600 daltons (Da) to optimize mass assignment, calibration, resolution, and sensitivity.

The kit contains calibration and test mixtures to:

- **Generate or verify mass calibration in MS mode** – The Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments calibration mixtures contain components with masses in the 900 Da to 3,600 Da mass range. Use these mixtures as the known masses to generate your calibration, or analyze them as unknowns to determine if your system is calibrated to the mass accuracy required by your application.
- **Evaluate and calibrate in MS/MS mode** – The calibration mixtures contain components you can evaluate for expected fragment ions and to calibrate AB SCIEX TOF/TOF™ Instruments.
- **Optimize and test sensitivity** – A low-concentration component (50 fmol/μL neurotensin) in the 1,000 to 2,000 Da mass range is provided to optimize and test sensitivity.
- **Test the setup, resolution, and function of the Timed Ion Selector (TIS) [Service Procedure only]** – The trypsin-digested beta-galactosidase can be used to confirm TIS function.

2 Materials

The Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments includes:

- **Empty vials with caps** – Six 0.5-mL vials
- **Matrix A:CHCA** – α-cyano-4-hydroxycinnamic acid, 2 vials (minimum 10 mg/vial)
- **Matrix A Diluent** – 50% acetonitrile in 0.1% TFA, 2 vials (2.5 mL/vial)

- **Standard Diluent** – 30% acetonitrile in 0.01% TFA, 2 vials (1 mL/vial)
- **Standard Mixtures** – Three, 1 vial each (see table below). Provided in lyophilized form that you reconstitute with 100 μL of Standard Diluent (you prepare Cal Mix 1 at two concentrations).

Table 1 Standard Mixture Components and Final Concentrations

Standard Mixture	Component	μg per Vial	Final Standard Concentrations (when mixed with matrix and diluent)
TOF/TOF Calibration Mixture	des-Arg ¹ -Bradykinin	2.3	1.0 pmol/μL
	Angiotensin I	6.5	2.0 pmol/μL
	Glu ¹ -Fibrinopeptide B	5.1	1.3 pmol/μL
	ACTH (1–17 clip)	10.5	2.0 pmol/μL
	ACTH (18–39 clip)	9.3	1.5 pmol/μL
	ACTH (7–38 clip)	27.5	3.0 pmol/μL
Calibration Mixture 1 (Cal Mix 1, 1:10)	des-Arg ¹ -Bradykinin	2.3	102 fmol/μL
	Angiotensin I	4.2	130 fmol/μL
	Glu ¹ -Fibrinopeptide B	5.1	130 fmol/μL
	Neurotensin	0.2	5 fmol/μL
Calibration Mixture 1 (Cal Mix 1, 1:100)	des-Arg ¹ -Bradykinin	2.3	10.2 fmol/μL
	Angiotensin I	4.2	13.0 fmol/μL
	Glu ¹ -Fibrinopeptide B	5.1	13.0 fmol/μL
	Neurotensin	0.2	0.5 fmol/μL
Beta-Galactosidase, Digested	Beta-Galactosidase, <i>Escherichia coli</i> , digested with bovine trypsin and lyophilized	72.8	250 fmol/μL

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3 Safety


Safety Alert Words

Four safety alert words appear in 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.

 **CAUTION** 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.

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

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

Chemical Hazard Warning

 **WARNING CHEMICAL HAZARD.** Some of the chemicals used with AB SCIEX instruments and protocols are potentially hazardous and can cause injury, illness, or death.

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 the MSDS for any chemical supplied by SCIEX. This service is free and available 24 hours a day.

To obtain MSDS go to www.sciex.com.

NOTE: For the MSDS of any chemical not distributed by SCIEX contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

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, and blood of humans and other animals have the potential to transmit infectious diseases. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4, <http://bmbi.od.nih.gov>)

- Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030, http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

Additional information about biohazard guidelines is available at: <http://www.cdc.gov>

Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.

4 Preparing Reagents

This section includes:

- Guidelines
- Preparing CHCA Matrix (Matrix A)
- Preparing TOF/TOF Cal Mix Stock Solution and Spotting Solution
- Preparing Cal Mix 1 Stock Solution and Spotting Solution
- Preparing Beta-Galactosidase (Digested) Stock Solution and Spotting Solution

4.1 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 – 1 μ L volumes, use a pipette with a volume range of 0.5 μ L – 2.0 μ L or 0.5 μ L – 2.5 μ L.

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

Determining the Needed Volume of Spotting Solution

The procedures in Section 4.3, Preparing TOF/TOF Cal Mix Stock Solution and Spotting Solution through Section 4.5, Preparing Beta-Galactosidase (Digested) Stock Solution and Spotting Solution yield 25 μ L of each spotting solution (matrix-standard mix), enough to spot approximately:

- 40 positions on a 192-well MALDI plate
- 24 positions on a 100-well MALDI plate


If you are a customer, 25 μ L is a sufficient volume for typical calibration, system optimization, and tuning procedures.


If you are an AB SCIEX service representative using this kit to perform the AB SCIEX Service Tuning Procedure, refer to the Service Tuning Procedure document to determine the volume of spotting solution needed.

Solution Stability

Solution	Stability Information
Reconstituted Matrix	<ul style="list-style-type: none"> Reconstitute immediately before use. Refrigerate, protected from light, when not in use.
Reconstituted Standard	<ul style="list-style-type: none"> Reconstitute immediately before use. Aliquot reconstituted standards in 5 – 10 μL volumes. Freeze unused aliquots for future use. Refrigerate when not in use. If you do not use a complete aliquot of reconstituted standard within 3 days, freeze. Do not repeatedly freeze and thaw. Doing so can cause the standard to degrade.

4.2 Preparing CHCA Matrix (Matrix A)

 **WARNING** **CHEMICAL HAZARD.** Matrix A Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **CAUTION** **CHEMICAL HAZARD.** Matrix A:(CHCA). The toxicological properties of this product have not been thoroughly investigated. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing CHCA Matrix (Matrix A)

To prepare CHCA Matrix (Matrix A) solution:

- Add 1,000 μ L of the Matrix A Diluent to the Matrix A:CHCA vial.

Important! The Matrix A Diluent vial contains more than 1,000 μ L of diluent. Do not add the entire contents of the Matrix A Diluent vial to the Matrix A vial.


- Vortex for 1 minute.
- Centrifuge for 1 minute to settle any undissolved matrix from the solution. Alternatively, allow the solution to sit for 5 minutes until undissolved matrix settles.

This vial contains the Matrix A solution for use in the following procedures. Use the supernatant (liquid with no precipitated solids) when instructed to add matrix.

4.3 Preparing TOF/TOF Cal Mix Stock Solution and Spotting Solution

Use the TOF/TOF Cal Mix spotting solution for high- and low-mass calibration and general tuning.


The following procedure yields a TOF/TOF Cal Mix spotting solution with the final concentrations indicated in Table 1 on page 1. For best results, reconstitute the standard immediately before use. For more information, see “Solution Stability” on page 3.

 **WARNING** **CHEMICAL HAZARD.** Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

TOF/TOF Proteomics Analyzer Calibration Mixture. The toxicological properties of this product have not been thoroughly investigated.

Standard Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.

Matrix A Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.

 **CAUTION** **CHEMICAL HAZARD.** **Matrix A:(CHCA).** The toxicological properties of this product have not been thoroughly investigated. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing TOF/TOF Cal Mix Stock Solution

1. Add 100 µL of Standard Diluent to the TOF/TOF Calibration Mixture vial.
2. Vortex the vial for at least 30 seconds.
3. Centrifuge the vial for 5 seconds.
4. Repeat step 2 and step 3.
5. Aliquot reconstituted standards in 5 – 10 µL volumes. Freeze unused aliquots for future use.

Preparing TOF/TOF Cal Mix Spotting Solution

1. Label a clean 0.5-mL vial with “TOF/TOF Cal Mix Spotting”.
2. If you are a customer, prepare 25 µL of spotting solution by adding the appropriate volumes of each solution listed in the table below to the “TOF/TOF Cal Mix Spotting” vial.

If you are an AB SCIEX service representative, prepare the volume of standard you need (see “Determining the Needed Volume of Spotting Solution” on page 3) by adding the appropriate volumes of each solution listed in the table below to the “TOF/TOF Cal Mix Spotting” vial.

TOF/TOF Cal Mix Spotting Solution Final Volume	Matrix A	Matrix A Diluent	TOF/TOF Cal Mix Stock Solution
25 µL	12 µL	12 µL	1 µL
50 µL	24 µL	24 µL	2 µL
75 µL	36 µL	36 µL	3 µL

3. Vortex the vial for at least 30 seconds.
4. Centrifuge the vial for 5 seconds.


5. Repeat step 3 and step 4.

4.4 Preparing Cal Mix 1 Stock Solution and Spotting Solution

Use the 1:10 dilution of Cal Mix 1 for low-mass calibration and sensitivity tuning.

Use the 1:100 dilution of Cal Mix 1 for MS/MS mode tuning.


The following procedures yield Cal Mix 1 spotting solutions with the final concentrations indicated in Table 1 on page 1. For best results, reconstitute the standard immediately before use. For more information, see “Solution Stability” on page 3.

 **WARNING** **CHEMICAL HAZARD.** Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Calibration Mixture 1. The toxicological properties of this product have not been thoroughly investigated.

Standard Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.

Matrix A Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.

 **CAUTION** **CHEMICAL HAZARD.** **Matrix A:(CHCA).** The toxicological properties of this product have not been thoroughly investigated. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing Cal Mix 1 Stock Solution

1. Add 100 µL of Standard Diluent to the Calibration Mixture 1 vial.
2. Vortex the vial for at least 30 seconds.
3. Centrifuge the vial for 5 seconds.
4. Repeat step 2 and step 3.
5. Aliquot reconstituted standards in 5 – 10 µL volumes. Freeze unused aliquots for future use.

Preparing Cal Mix 1 Spotting Solution (1:10)

1. Label a clean 0.5-mL vial with “Cal Mix 1:10 Dilution”, then add:
 - 9 µL of Standard Diluent
 - 1 µL of the Cal Mix 1 stock solution (prepared above)
2. Vortex the vial for at least 30 seconds.
3. Centrifuge the vial for 5 seconds.
4. Repeat step 2 and step 3.
5. Label a clean 0.5-mL vial with “Cal Mix Spotting (1:10)”.

- If you are a customer, prepare 25 µL of spotting solution by adding the appropriate volumes of each solution listed in the table below to the “Cal Mix Spotting (1:10)” vial.

If you are an AB SCIEX service representative, prepare the volume of standard you need (see “Determining the Needed Volume of Spotting Solution” on page 3) by adding the appropriate volumes of each solution listed in the table below to the “Cal Mix 1:10 Spotting” vial.

Cal Mix 1 Spotting Solution (1:10) Final Volume	Matrix A	Matrix A Diluent	Cal Mix 1 (1:10) Dilution
25 µL	10 µL	14 µL	1 µL
50 µL	20 µL	28 µL	2 µL
75 µL	30 µL	42 µL	3 µL

Note: If you do not observe satisfactory sensitivity with the Cal Mix spotting solution, you can prepare more spotting solution and change the ratio of volumes of Matrix A to Matrix A Diluent, without changing the total volume of matrix and diluent. Changing the ratio of matrix to diluent affects the concentration of matrix, not the concentration of standard. For example, instead of the 10:14:1 volumes given above, you could use 8:16:1 or 12:12:1. (Do not change the volume of standard.)

- Vortex the vial for at least 30 seconds.
- Centrifuge the vial for 5 seconds.
- Repeat step 7 and step 8.

Preparing Cal Mix 1 Spotting Solution (1:100)

- Label a clean 0.5-mL vial with “Cal Mix 1:100 Dilution”, then add:
 - 99 µL of Standard Diluent
 - 1 µL of the Cal Mix 1 stock solution (prepared in “Preparing Cal Mix 1 Stock Solution” on page 4)
- Vortex the vial for at least 30 seconds.
- Centrifuge the vial for 5 seconds.
- Repeat step 2 and step 3.
- Label a clean 0.5-mL vial with “Cal Mix Spotting (1:100)”.
- If you are a customer, prepare 25 µL of spotting solution by adding the appropriate volumes of each solution listed in the table below to the “Cal Mix Spotting (1:100)” vial.

If you are an AB SCIEX service representative, prepare the volume of standard you need (see “Determining the Needed Volume of Spotting Solution” on page 3) by adding the appropriate volumes of each solution listed in the table below to the “Cal Mix 1:100 Spotting” vial.

Cal Mix 1 Spotting Solution (1:100) Final Volume	Matrix A	Matrix A Diluent	Cal Mix 1 (1:100) Dilution
25 µL	10 µL	14 µL	1 µL
50 µL	20 µL	28 µL	2 µL
75 µL	30 µL	42 µL	3 µL

Note: If you do not observe satisfactory sensitivity with the Cal Mix spotting solution, you can prepare more spotting solution and change the ratio of volumes of Matrix A to Matrix A Diluent, without changing the total volume of matrix and diluent. Changing the ratio of matrix to diluent affects the concentration of matrix, not the concentration of standard. For example, instead of the 10:14:1 volumes given above, you could use 8:16:1 or 12:12:1. (Do not change the volume of standard.)

- Vortex the vial for at least 30 seconds.
- Centrifuge the vial for 5 seconds.
- Repeat step 7 and step 8.

4.5 Preparing Beta-Galactosidase (Digested) Stock Solution and Spotting Solution

Use the Beta-Galactosidase (Digested) spotting solution to confirm Timed Ion Selector (TIS) function, peptide identification accuracy, or MS/MS mode mass accuracy.

The following procedure yields a Beta-Galactosidase spotting solution with the final concentration indicated in Table 1 on page 1. For best results, reconstitute the standard immediately before use. For more information, see “Solution Stability” on page 3.



WARNING **CHEMICAL HAZARD.** Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Beta-galactosidase, digested. The toxicological properties of this product have not been thoroughly investigated.

Standard Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.

Matrix A Diluent (containing acetonitrile) is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and damage to the heart, blood system, liver, and kidneys.



CAUTION **CHEMICAL HAZARD. Matrix A:(CHCA).** The toxicological properties of this product have not been thoroughly investigated. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing Beta-Galactosidase Stock Solution

- Add 100 µL of Standard Diluent to the Beta-Galactosidase vial.
- Vortex the vial for at least 30 seconds.
- Centrifuge the vial for 5 seconds.
- Repeat step 2 and step 3.
- Aliquot reconstituted standards in 5 – 10 µL volumes. Freeze unused aliquots for future use.

Preparing Beta-Galactosidase Spotting Solution

1. Label a clean 0.5-mL vial with "Beta Gal Spotting".
2. If you are a customer, prepare 25 µL of spotting solution by adding the appropriate volumes of each solution listed in the table below to the "Beta Gal Spotting" vial.

If you are an AB SCIEX service representative, prepare the volume of standard you need (see "Determining the Needed Volume of Spotting Solution" on page 3) by adding the appropriate volumes of each solution listed in the table below to the "Beta Gal Spotting" vial.

Beta-Gal Spotting Solution Final Volume	Matrix A	Matrix A Diluent	Beta-Gal Stock Solution
25 µL	12 µL	12 µL	1 µL
50 µL	24 µL	24 µL	2 µL
75 µL	36 µL	36 µL	3 µL

3. Vortex the vial for at least 30 seconds.
4. Centrifuge the vial for 5 seconds.
5. Repeat step 3 and step 4.

5 Spotting Plates

Recommended Pipette Volume Ranges

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

Positions to Spot

If you are a customer, the number of positions to spot depends on the tuning procedure you are performing. It is good practice to spot approximately four positions of the standards you use in the center of the plate. For example, spot four positions of one standard in the center of row D, four positions of another standard in the center of row E, and so on.

If you are an AB SCIEX service representative, refer to the AB SCIEX Service Tuning Procedure document to determine the positions to spot.

Spotting

To spot a plate with spotting solution:

1. Aspirate the appropriate volume of supernatant from each spotting solution vial:
 - **192-well plates** – 0.5 µL
 - **100-well plates** – 1.0 µL
2. Dispense the matrix-standard spotting solution on the sample plate. Do not touch the tip of the pipette to the surface of the plate. Touching can cause uneven crystallization.
3. Allow the mixture to air dry until all solvent is evaporated, usually less than 5 minutes.

Analyze within one day for best results.

6 Analyzing Standards

Default Methods Provided With Your System

Your 4000 Series Explorer™ Software includes default methods that are useful as starting points for creating methods to analyze the standards in the Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments. Default methods have typical parameter values for different operating modes and mass ranges. Default methods are available by selecting **File>New>Methods** in the 4000 Series Explorer Software.

Analyzing Standards

Analyze the standards according to the needs of your application.

For representative spectra and mass assignments for standards and matrixes in the kit, refer to:

- Section 7, TOF/TOF Cal Mix and Cal Mix 1 Spectra and Masses
- Section 8, Beta-Galactosidase Spectra and Masses

7 TOF/TOF Cal Mix and Cal Mix 1 Spectra and Masses

This section includes:

- Linear Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1
- Reflector Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1
- MS/MS Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1
- Mass Assignments for CHCA Matrix A, TOF/TOF Cal Mix, Cal Mix 1

Masses are included in the spectra for peak identification only. Use the precise masses listed in Table 2 on page 10 for calibration.

Note: If you do not observe satisfactory sensitivity with the Cal Mix (1:10) or Cal Mix (1:100) spotting solutions, you can prepare more spotting solution and change the ratio of volumes of Matrix A and Matrix A Diluent. Refer to "Preparing Cal Mix 1 Spotting Solution (1:10)" on page 4 and "Preparing Cal Mix 1 Spotting Solution (1:100)" on page 5.

7.1 Linear Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1

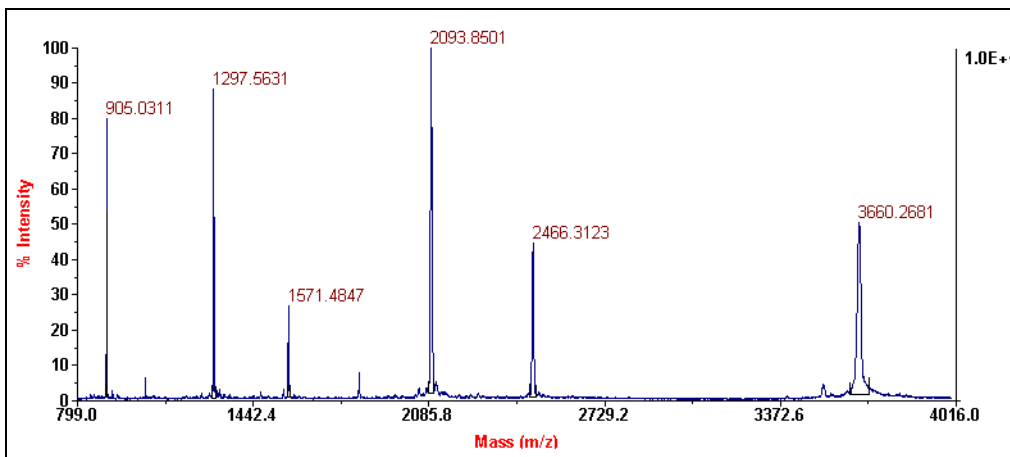


Figure 1 TOF/TOF Calibration Mixture, Linear Mode

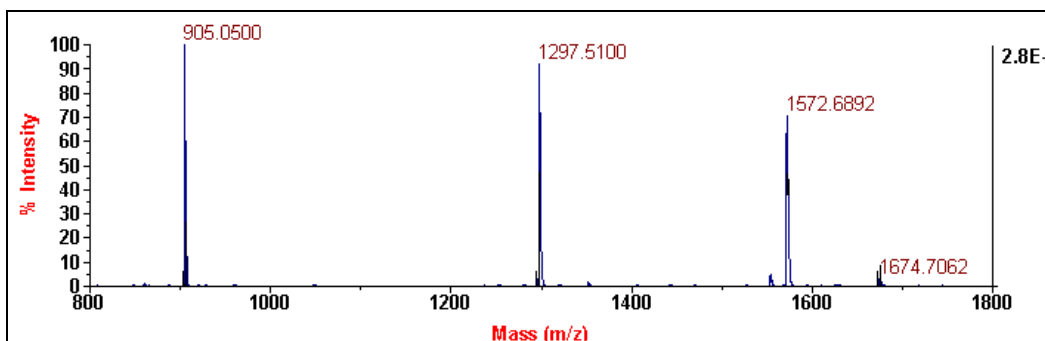


Figure 2 Calibration Mixture 1 (1:10 Dilution), Linear Mode

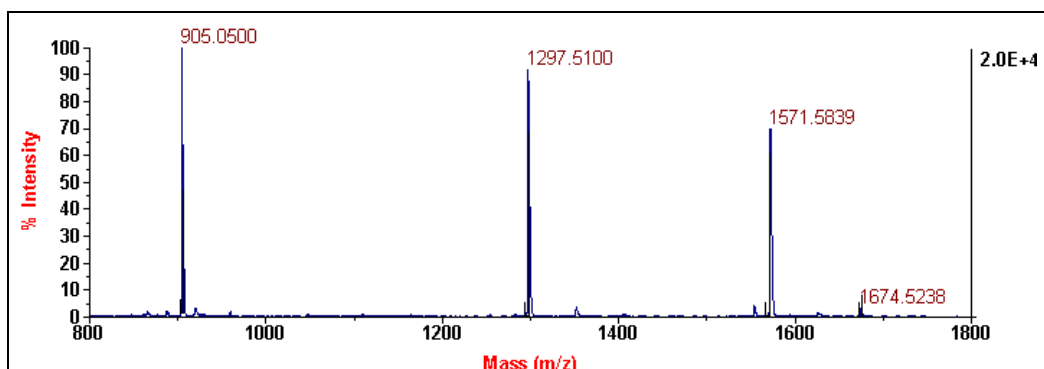


Figure 3 Calibration Mixture 1 (1:100 Dilution), Linear Mode

7.2 Reflector Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1

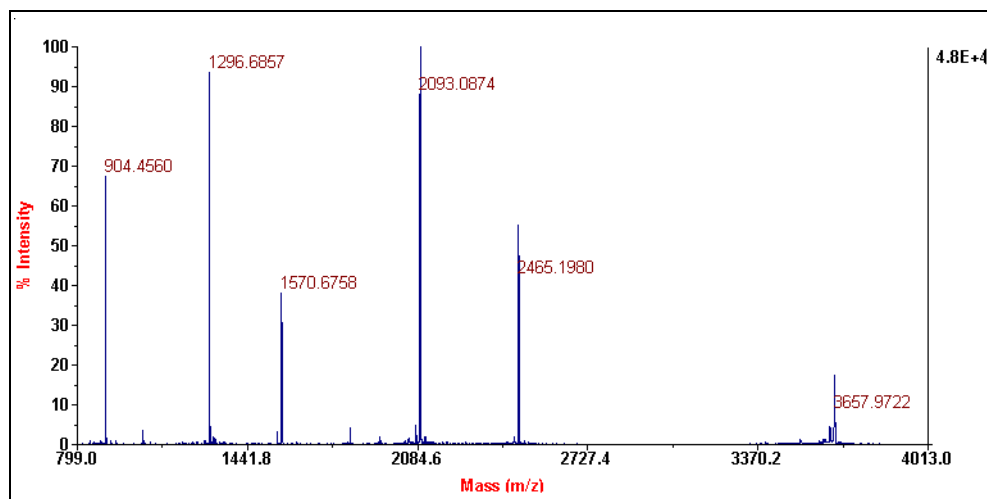


Figure 4 TOF/TOF Calibration Mixture, Reflector Mode

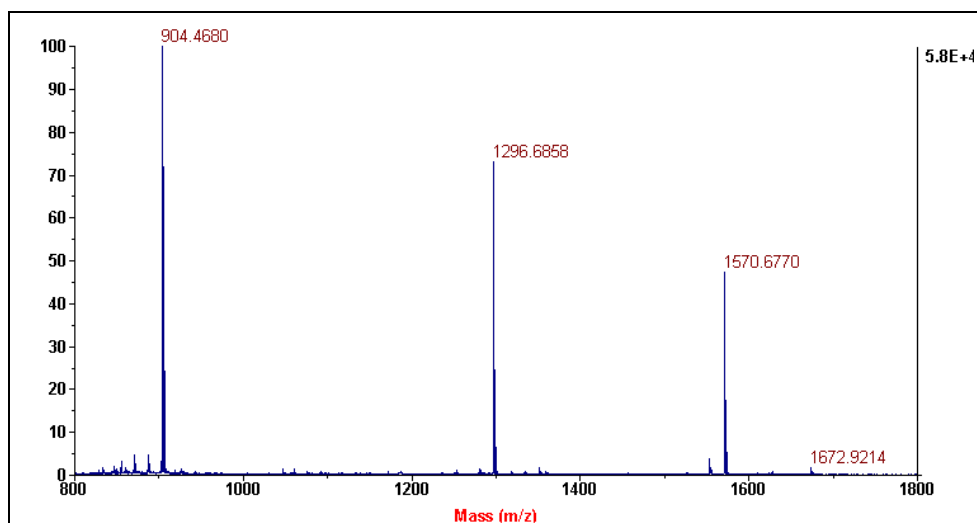


Figure 5 Calibration Mixture 1 (1:10 Dilution), Reflector Mode

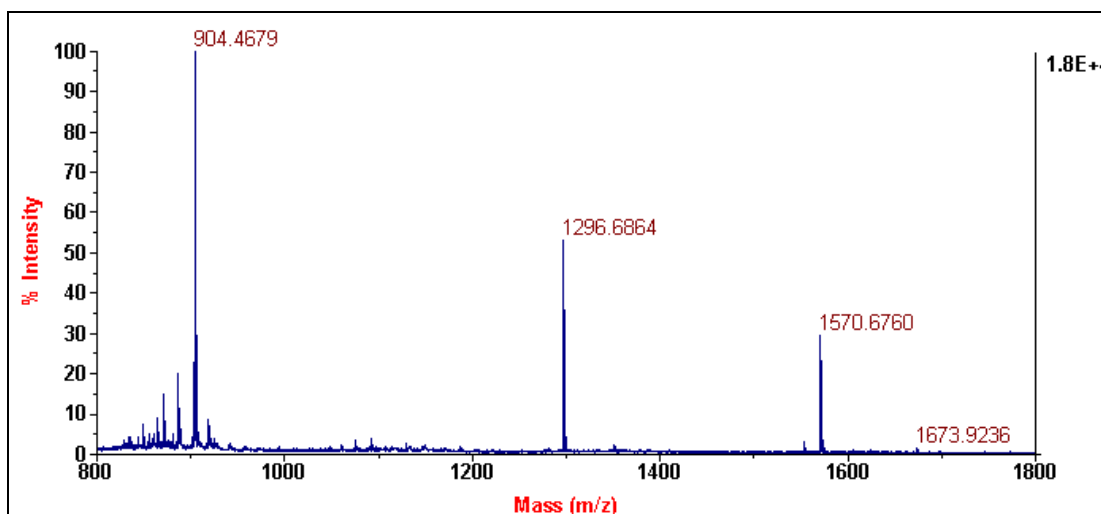


Figure 6 Calibration Mixture 1 (1:100 Dilution), Reflector Mode

7.3 MS/MS Mode Spectra for TOF/TOF Cal Mix and Cal Mix 1

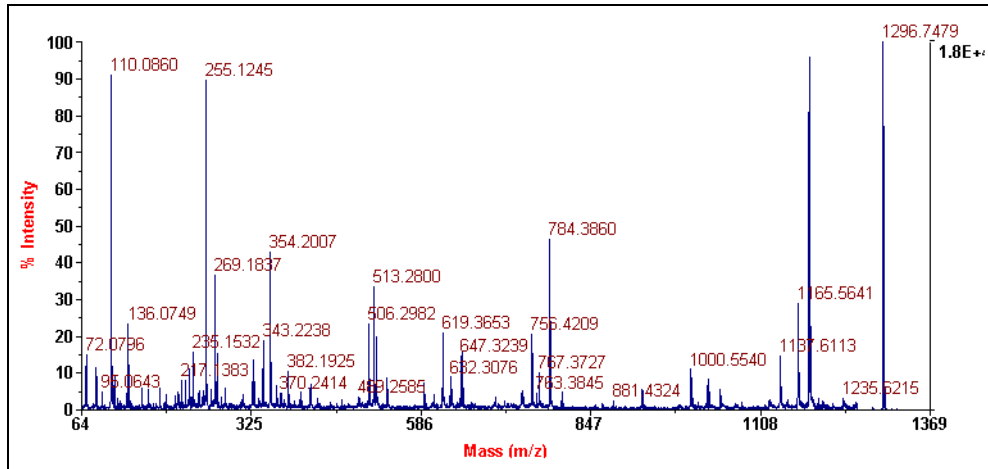


Figure 7 TOF/TOF Calibration Mixture, MS/MS Mode Angiotensin I (m/z 1,296.68)

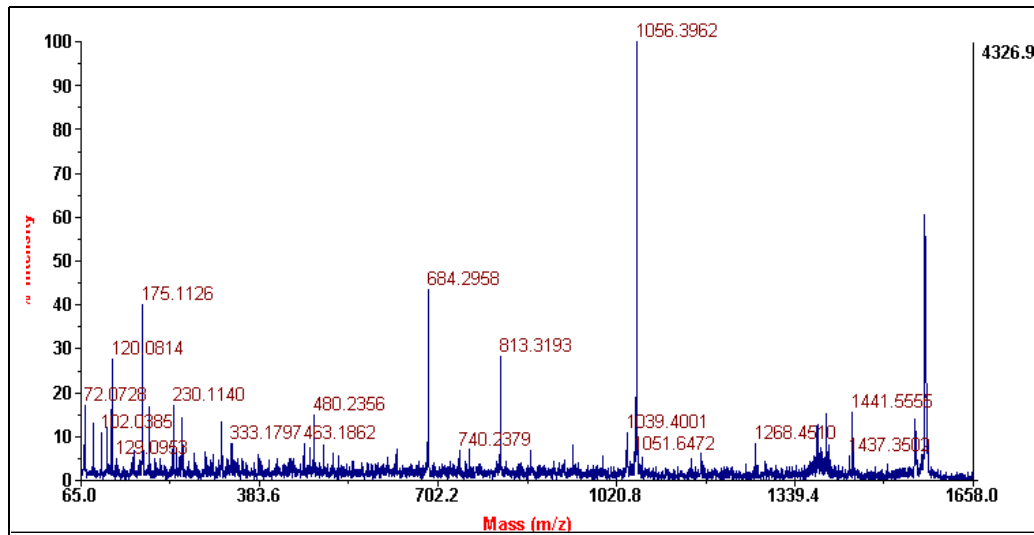


Figure 8 Calibration Mixture 1 (1:100 Dilution), MS/MS Mode Glu¹-Fibrinopeptide B (m/z 1,570)

7.4 Mass Assignments for CHCA Matrix A, TOF/TOF Cal Mix, Cal Mix 1

Use the masses listed in Table 2 for calibration.

Table 2 Mass Assignments for Standards and Matrices

Component	Charge (n)	(M+nH) ⁿ⁺ Average (Da)	(M+nH) ⁿ⁺ Monoisotopic (Da)
Matrix			
CHCA matrix	+1	190.18	190.0504
CHCA matrix (dimer)	+1	379.35	379.0930
TOF/TOF Calibration Mixture			
des-Arg ¹ -Bradykinin	+1	905.05	904.4681
Angiotensin I	+1	1,297.51	1,296.6853
Glu ¹ -Fibrinopeptide B	+1	1,571.61	1,570.6774
ACTH (clip 1–17)	+1	2,094.46	2,093.0867
ACTH (clip 18–39)	+1	2,466.72	2,465.1989
ACTH (clip 7–38)	+1	3,660.19	3,657.9294
Calibration Mixture 1			
des-Arg ¹ -Bradykinin	+1	905.05	904.4681
Angiotensin I	+1	1,297.51	1,296.6853
Glu ¹ -Fibrinopeptide B	+1	1,571.61	1,570.6774
Neurotensin	+1	1,673.96	1,672.9175

8 Beta-Galactosidase Spectra and Masses

8.1 Tryptic Digest Spectrum

The tryptic digest spectrum for beta-galactosidase is shown in Figure 9.

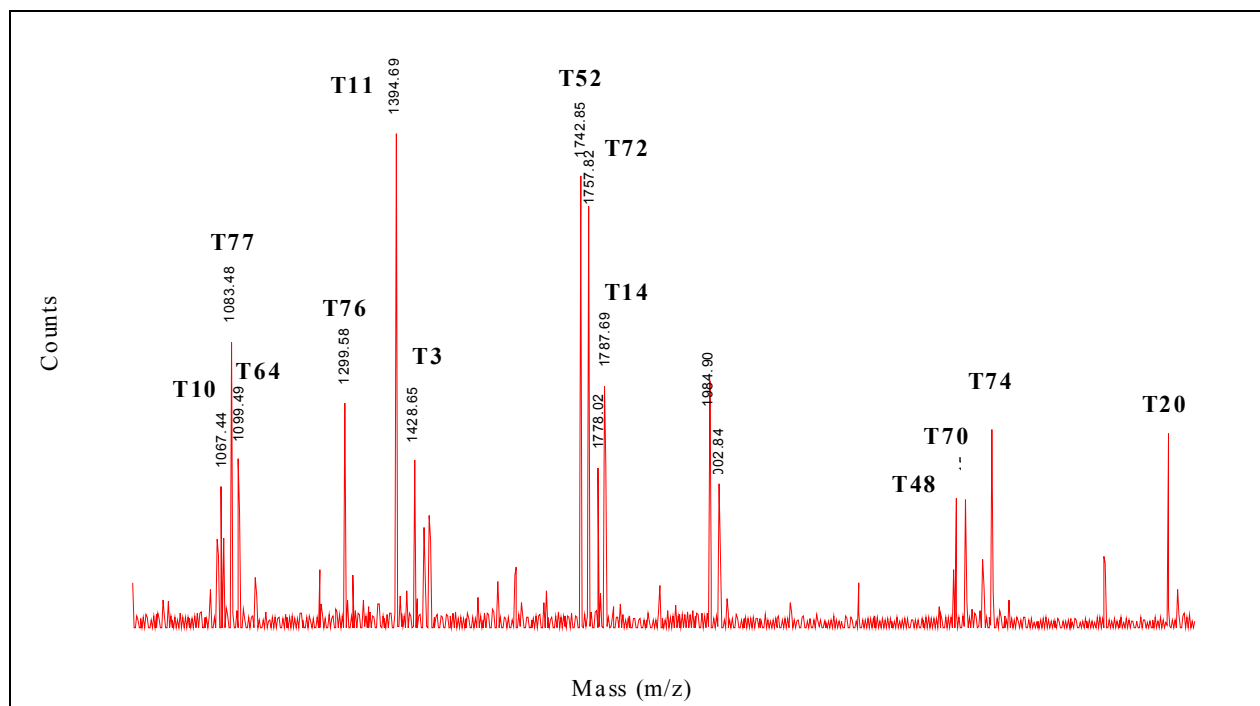


Figure 9 Beta-Galactosidase Tryptic Digest Spectrum

8.2 Peptide Mass Assignments

The peptide mass assignments for beta-galactosidase are listed in Table 3.

Table 3 Beta-Galactosidase Peptide Mass Assignments

Fragment Number	Peptide Fragment	Residues (Start-End)	(M+H) ⁺ Monoisotopic, Calculated (Da)
T10	WVGYGQDSR	159–167	1,067.4910
T77	GDFQFNISR	954–962	1,083.5223
T64	IDPNAWVER	802–810	1,099.5536
T76	ELNYGPHQWR	944–953	1,299.6234
T11	LPSEFDLSAFLR	168–179	1,394.7320
T3	DWENPGVTQLNR	16–27	1,428.6872
T52	LSGQTIEVTSEYLFR	632–646	1,742.8965
T72	VNWLGLGPQENYPDR	896–910	1,757.8611
T14	WSDGSYLEDQDMWR	192–205	1,787.7335
T48	YDENGNPWSAYGGDFGDTPNDR	579–600	2,446.9812
T70	IDGSGQMAITVDVEASDTPHAR	859–882	2,466.1935
T74	WDLPLSDMYTPYVFPSENGLR	919–939	2,516.1808
T20	VTVSLWQGETQVASGTAPFGGEIIDER	257–283	2,847.4165

9 Storing the Kit

For best results, reconstitute the standards immediately before use. For more information, see "Solution Stability" on page 3. Store the Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments 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
Reconstituted standards	-20 °C	6 months (If maintained at -20 °C continually. Freezing and thawing repeatedly can cause the standard solution to degrade.)
Reconstituted standards	4 °C	3 days
Reconstituted matrix	4 °C	1 week

10 Accessories, Spare Parts, and Ordering Information

Item	Quantity	Part Number
TOF/TOF Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments	1 kit	4333604

11 Technical Support

AB SCIEX is committed to meeting the needs of your research through enabling technologies like the Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments. Our dedicated support staff is available to answer questions about using this product to the fullest extent possible.

AB SCIEX offers a complete line of time-of-flight (TOF) mass spectrometry products to meet your analysis needs. Please contact your AB SCIEX representative for technical and ordering information.

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

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