Applied Biosystems

Sequazyme™ Peptide Mass Standards Kit

Operating Instructions

1 Product Description

The Sequazyme™ Peptide Mass Standards Kit includes reagents needed to test instrument function, optimize instrument parameters, and calibrate the mass scale using known peptide standards. The Sequazyme™ Peptide Mass Standards Kit is designed for use with the Voyager™ Biospectrometry™ Workstation or any MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry system.

Standards in the kit require minimal preparation and cover a wide mass range. The standards for this kit were chosen on the basis of high purity and consistent results when analyzed using MALDI-TOF mass spectrometry.

Applications

Using the mixtures of known peptides and proteins in the Sequazyme Peptide Mass Standards Kit, you can monitor results in a mass range from 900 to 17,000 daltons (Da) for mass assignment, resolution, and sensitivity.

This kit provides the necessary experimental information to determine instrument parameters used in optimizing calibration, resolution, sensitivity, post-source decay (PSD) analysis, and collision-induced dissociation (CID) analysis.

The kit contains calibration and test mixtures you can use for the following:

- **Generate or verify mass calibration**—Calibration Mixtures 1, 2, and 3 include component masses in a mass range from 900 Da to 17,000 Da. Use these mixes 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.
- **Optimize and test sensitivity**—Calibration Mixture 1 also contains a low-concentration component (50 fmol/µl of Neurotensin) in the 1,000 to 2,000 Da mass range.
- **Test the setup, resolution, and function of the Timed Ion Selector (TIS)**—The TIS Test Mixture contains 3 peptides with mass differences of 25 Da or less. Set the Precursor Ion Mass to one of the three masses and make sure that the other two masses are screened out by the Timed Ion Selector on a Voyager system with a reflector detector.
- **Evaluate and calibrate in PSD or CID modes**—The PSD and CID Test Standards contain single components you can use to evaluate for expected fragment ions, and to calibrate Voyager systems with reflector detectors in PSD and CID modes.

2 Materials

The Sequazyme™ Peptide Mass Standards Kit includes:

Matrices and diluents

- **Matrix A**:**CHCA**—α-cyano-4-hydroxycinnamic acid, 2 vials (5–8 mg/vial)
- **Matrix B:Sinapinic Acid**—3,5-Dimethoxy-4-hydroxycinnamic acid, 2 vials (7–10 mg/vial)
- **Matrix A Diluent**—50% acetonitrile in 0.3% TFA, 2 vials (1 ml/vial)
- **Matrix B Diluent**—30% acetonitrile in 0.3% TFA, 2 vials (1 ml/vial)
- **Standard Diluent**—30% acetonitrile in 0.01% TFA, 1 vial (1 ml/vial)
- **Empty vials with caps**—Ten 0.5-ml vials

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Standards

Standards are provided in lyophilized form and must be reconstituted with 100 µl of Standard Diluent.

Standard	Component	Weight (µg) per Vial	Final Concentration (when mixed with matrix)
Calibration Mixture 1	des-Arg^1-Bradykinin	2.3	1.0 pmol/µl
	Angiotensin I	4.2	1.3 pmol/µl
	Glu^1-Fibrinopeptide B	5.1	1.3 pmol/µl
	Neurotensin	0.2	50 fmol/µl
Calibration Mixture 2	Angiotensin I	6.5	2.0 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
	Insulin, bovine	50.2	3.5 pmol/µl
Calibration Mixture 3	Insulin, bovine	7.1	0.5 pmol/µl
	Thioredoxin, E. coli	80.0	2.75 pmol/µl
	Apomyoglobin, horse	170.0	4.0 pmol/µl
TIS Test Mixture	Substance P-amide	6.9	2.0 pmol/µl
	Pro^9-Substance P-amide	6.9	2.0 pmol/µl
	Tyr^8-Substance P-amide	6.9	2.0 pmol/µl
PSD Test Standard	Angiotensin I	6.5	2.0 pmol/µl
CID Test Standard	Substance P-amide	6.9	2.0 pmol/µl

3 Preparing Matrix and Standards

See Section 7, Storing the Kit, for storage and stability conditions of prepared reagents.

After you use up the matrices provided in the kit, you can use commercially available CHCA and sinapinic acid matrices to analyze standards.

WARNING. CHEMICAL HAZARD. Diluents (with acetonitrile) *are flammable liquids and vapors. They may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION. CHEMICAL HAZARD. Alpha-cyano-4-hydroxycinnamic acid (CHCA) *may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION. CHEMICAL HAZARD. Sinapinic acid *may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION. CHEMICAL HAZARD. Calibration Mix 1 *may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

WARNING. CHEMICAL HAZARD. Calibration Mix 2 *may cause an allergic skin and respiratory tract reaction. Exposure may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION. CHEMICAL HAZARD. Calibration Mix 3 *may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CAUTION. CHEMICAL HAZARD. TIS Test Mixture *may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Important. PSD Test Standard. *Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

Important. CID Test Standard. *Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.*

CHCA matrix

Use CHCA matrix for all standards *except* Calibration Mixture 3.

To prepare CHCA matrix:

1. Add the entire contents of the **Matrix A Diluent** vial to the **Matrix A:CHCA** vial.
2. Vortex for 1 minute.
3. Centrifuge for 20 seconds to remove any undissolved matrix from the solution. Alternatively, allow the solution to sit for 5 minutes until undissolved matrix settles.

   Use the supernatant for sample preparation.

Sinapinic acid matrix

Use sinapinic acid matrix for Calibration Mixture 3.

To prepare sinapinic acid matrix:

1. Add the entire contents of the **Matrix B Diluent** vial to the **Matrix B:Sinapinic Acid** vial.
2. Vortex for 1 minute.
3. Centrifuge for 20 seconds to remove any undissolved matrix from the solution. Alternatively, allow the solution to sit for 5 minutes until undissolved matrix settles.

   Use the supernatant for sample preparation.

Standards

The final concentration for each standard after mixing with matrix is listed in Section 2, Materials.

To prepare the standards:

1. Add 100 µl of Standard Diluent to each of the six standard vials.
2. Vortex standard vials.
3. Add 24 µl of the appropriate reconstituted matrix to an empty 0.5-ml microcentrifuge tube:
   - **CHCA matrix**—Calibration mixture 1 and 2, PSD Test Standard, CID Test Standard, TIS Test Mixture
   - **Sinapinic Acid matrix**—Calibration mixture 3
4. Add 1 µl of the desired standard stock solution to the 0.5 ml microcentrifuge tube. Place the pipet tip into the matrix, then dispense the standard to ensure mixing.
5. Vortex briefly at low speed.

4 Loading Matrix:Standard on Sample Plates

Each matrix:standard preparation yields 25 µl, enough to load approximately 25 sample positions.

To load a plate:

1. Load 0.5 to 1.0 µl of premixed matrix:standard solution on the sample plate.
2. Allow the mixture to air dry until all solvent is evaporated, usually less than 5 minutes.

   Analyze within one day for best results.

5 Analyzing Standards

Standard methods on your system

Your Voyager workstation includes standard methods created during factory testing of your instrument. Standard methods contain typical parameters for a given mass or mass range. Standard methods are useful as a starting point when creating methods to analyze the standards in the Peptide Mass Standards Kit.

Analyzing standards

Analyze the standards according to the needs of your application.

Refer to Section 6, Peptide Standard Spectra and Masses, for representative spectra and mass assignments for standards and matrices in the kit.

6 Peptide Standard Spectra and Masses

The following figures show representative spectra for test mixtures. Masses are included in the spectra for peak identification only. Use the precise masses listed in Table 1 on page 6 for calibration.

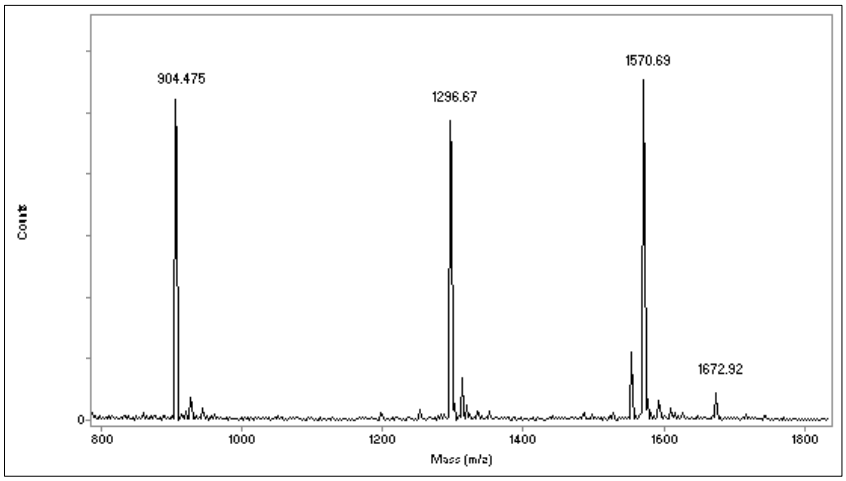

Figure 1 Calibration Mixture 1, Linear Mode

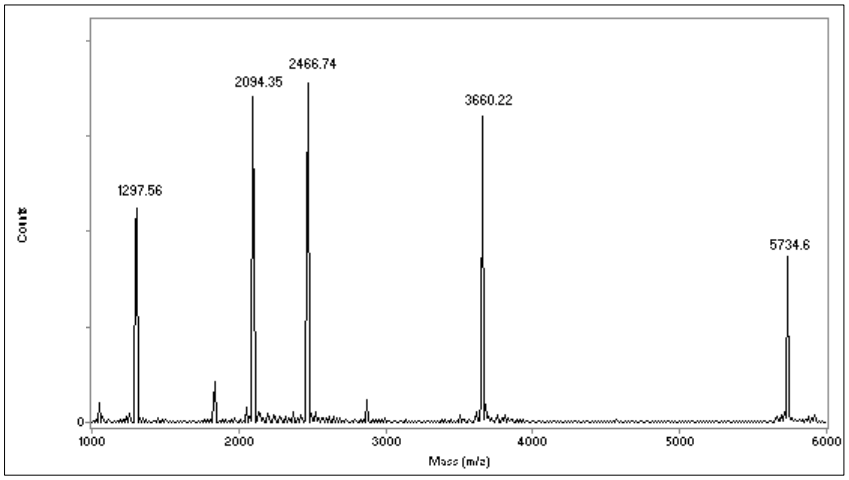

Figure 2 Calibration Mixture 2, Linear Mode

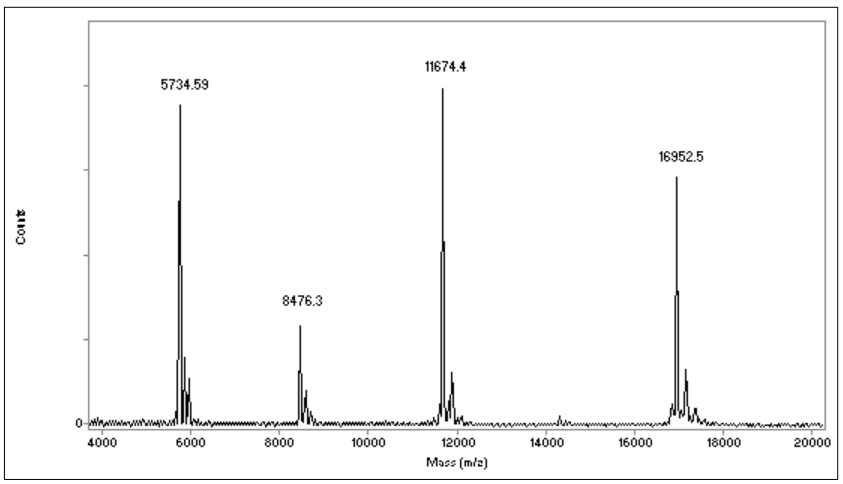

Figure 3 Calibration Mixture 3, Linear Mode

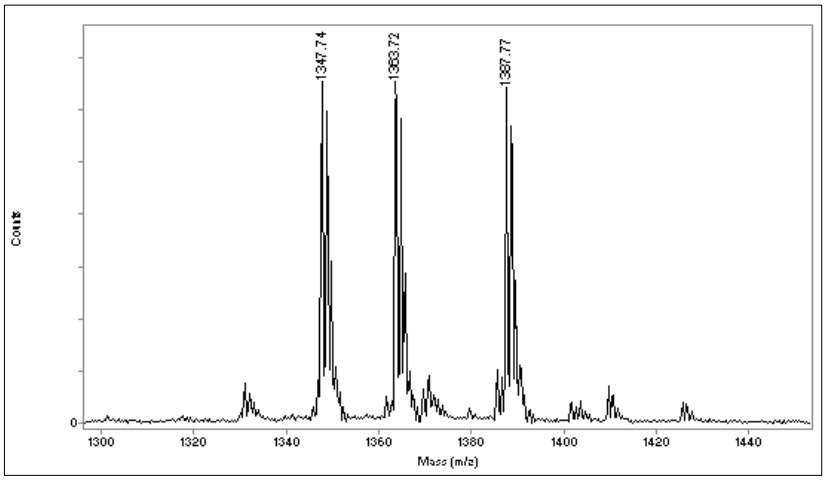

Figure 4 TIS Test Mixture, Reflector Mode

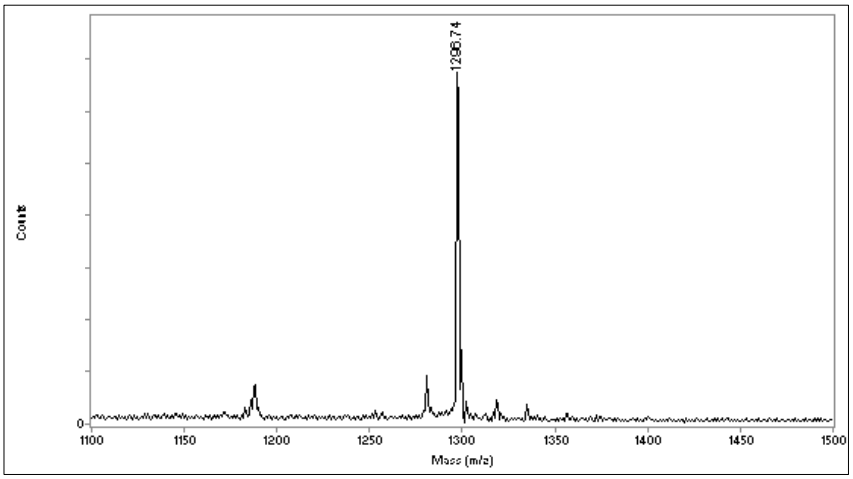

Figure 5 PSD Test Standard, PSD Mode

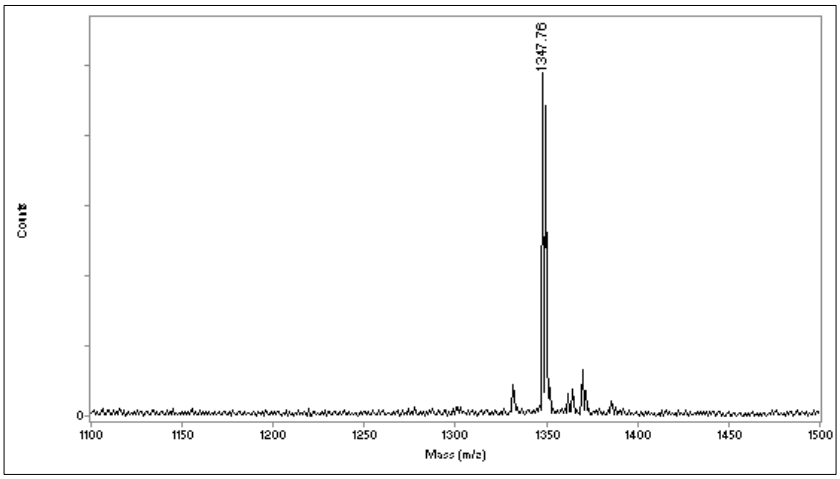

Figure 6 CID Test Standard, PSD Mode

Use the masses listed in Table 1 for calibration.

Table 1 Mass Assignments for Standards and Matrices

Name	Charge (n)	$(M+nH)n^+$ Average	$(M+nH)n^+$ Monoisotopic
Matrix			
CHCA matrix	+1	190.18	190.0504
CHCA matrix (dimer)	+1	379.35	379.0930
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
Calibration Mixture 2			
Angiotensin I	+1	1,297.51	1,296.6853
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
Insulin (bovine)	+1	5,734.59	5,730.6087
	+2	2,867.80	2,865.8083
Calibration Mixture 3			
Insulin (bovine)	+1	5,734.59	—
	+2	2,867.80	—
Thioredoxin (E. coli)	+1	11,674.48	—
	+2	5,837.74	—
Apomyoglobin (horse)	+1	16,952.56	—
	+2	8,476.78	—
PSD Test Standard			
Angiotensin I	+1	1,297.51	1,296.6853
CID Test Standard			
Substance P-amide	+1	1,348.66	1,347.7360
TIS Test Mixture			
Substance P-amide	+1	1,348.66	1,347.7360
Tyr^8-Substance P-amide	+1	1,364.66	1,363.7309
Pro^9-Substance P-amide	+1	1,388.73	1,387.7673

7 Storing the Kit

Store the Sequazyme Peptide 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
Reconstituted standards	–20°C	6 months
Reconstituted matrix	4°C	1 week

8 Accessories, Spare Parts, and Ordering Information

Item	Quantity	Part Number
Sequazyme Peptide Mass Standards Kit	1 kit	P2-3143-00
BSA Test Standard Kit	1 kit	2-2158-00
IgG Mass Standard Kit	1 kit	GEN602151
Sequazyme Peptide Mass Standards Install Kit, includes: • Sequazyme Peptide Mass Standards Kit • BSA Test Standard Kit • IgG Mass Standard Kit	1 kit	4316866

9 Technical Support

Applied Biosystems is committed to meeting the needs of your research through enabling technologies like the Sequazyme Peptide 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 complete line of time-of-flight (TOF) mass spectrometry products to meet your analysis needs. Please contact your Applied Biosystems representative for technical and ordering information.

Applied Biosystems publishes a continuing series of Application Notes and Technical Notes highlighting ladder sequencing applications and strategies. For a publications list, or for further details or answers to questions related to other products, contact Applied Biosystems using the information on the last page.

Products and procedures described in this protocol are intended for research purposes only. Not for use in diagnostic procedures.

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