

Operating Instructions

1 Product Description

Use the Sequazyme™ IgG1 Mass Standard Kit to test instrument function, optimize instrument parameters, and calibrate the mass scale using a known standard. The kit is designed for use with the Sequazyme™ Peptide Mass Standards Kit with the Voyager™ Biospectrometry™ Workstation or with any MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry system.

The standard in the kit requires minimal preparation and covers a mass range of 100,000 to 200,000 daltons (Da). The standard was chosen for this kit on the basis of high purity and consistent results when analyzed using MALDI-TOF mass spectrometry.

Applications

Using the Sequazyme IgG1 Mass Standard Kit, you can monitor results in a mass range from 100,000 to 200,000 Da for mass assignment, resolution, and sensitivity.

2 Materials

Materials Provided

The Sequazyme IgG1 Mass Standard Kit includes—lyophilized mouse immunoglobulin 1, **IgG1** (1 vial, 0.05 mg/vial)

Matrix and Diluent

- **Matrix B:Sinapinic Acid**—3,5-Dimethoxy-4-hydroxycinnamic acid, 2 vials (7 to 10 mg/vial)
- **Matrix B Diluent**—30% acetonitrile in 0.3% TFA, 2 vials (1 ml/vial)

After you use up the sinapinic acid matrix provided in the Sequazyme Peptide Mass Standards Kit, you can use commercially available sinapinic acid to analyze standards.

3 Preparing Matrix and Standards

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

⚠ WARNING CHEMICAL HAZARD. Diluent (with acetonitrile) is a flammable liquid and vapor. It 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. 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.

Reconstitute IgG1

Store the Sequazyme IgG1 Mass Standard at –20° C prior to use. Sinapinic Acid Matrix from a Sequazyme kit is required to use this standard.

1. Add 50 µl HPLC-grade water to the **IgG1** vial.
2. Recap and vortex for 1 minute.

You can use the standard immediately. Store at –20° C after use.

Sinapinic Acid Matrix

Use sinapinic acid matrix for Calibration Mixture 3.

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1. Add the entire contents of the **Matrix B Diluent** vial to the **Matrix B:Sinapinic Acid** vial.
2. Vortex for 1 minute.
3. Warm the solution, if necessary, to completely dissolve the solid.
Use the supernatant for sample preparation.

Standard

Final concentration of the standard after mixing with matrix is 0.6 pmol/ μ l.

1. Pipette 1 μ l of the reconstituted IgG1 solution into a microcentrifuge tube.
2. Add 10 μ l of the sinapinic acid matrix.
3. Vortex briefly at low speed.

4 Loading Matrix:Standard on Sample Plates

Each matrix:standard preparation yields 11 μ l, enough to load approximately 11 sample positions.

1. Pipette 1.0 μ l of sample on the MALDI 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 starting points when you create methods to analyze the standard in the Sequazyme IgG1 Mass Standard Kit.

Analyzing standards

Analyze the standard according to the needs of your application.

Refer to Section 6, IgG1 Standard Spectrum and Masses, for representative spectra and mass assignments for standards and matrices in the kit.

6 IgG1 Standard Spectrum and Masses

Figure 1 shows a representative spectrum for the test mixture. Masses are included in the spectrum for peak identification only. Use the precise masses listed in Table 1 for calibration.

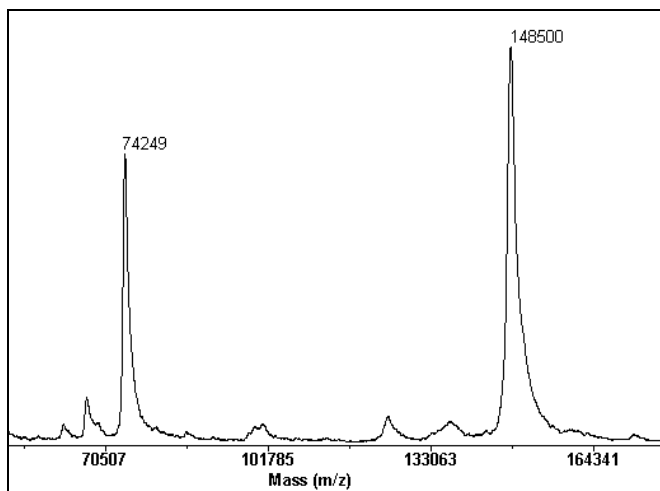


Figure 1 IgG1 Linear Resolution

Table 1 Mass Assignments for the Standard

Standard	Charge (n)	(M+nH) ⁺ Average
IgG1	+1	148,500
	+2	74,249

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

Description	Quantity (Kits)	Part Number
Sequazyme™ Peptide Mass Standards Install Kit includes: <ul style="list-style-type: none">Sequazyme Peptide Mass Standards KitBSA Test Standard KitIgG1 Mass Standard Kit	1	4316866
Sequazyme Peptide Mass Standards Kit	1	P2-3143-00
BSA Test Standard Kit	1	2-2158-00
IgG1 Mass Standard Kit	1	GEN602151

9 Technical Support

Applied Biosystems is committed to meeting the needs of your research through enabling technologies like the Sequazyme IgG1 Mass Standard 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. Please contact Applied Biosystems directly for a publication list.

For further details or for answers to questions related to other products, contact Applied Biosystems. Refer to the back page for contact information.

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