MALDI Calibration with SpheriCal® Aqua Peptide Low

Thank you for making SpheriCal®Aqua your choice of calibrant. You will come to find that it outperforms comparable calibrants available on the market and that it offers you the precision needed by today's MALDI-TOF MS instruments. The following is a short and simple explanation on how to set up the necessary files as well as a suggestion for sample preparation for the best possible calibration of your MALDI-ToF. It is written to comply with Bruker instruments but is applicable to other instruments – please adapt to your software and hardware.

1. Product Description and Storage information

SpheriCal® Aqua Peptide Low is a mixture of six monodisperse dendrimers which is intended for m/z calibration of MALDI-TOF mass spectrometers. This product is intended for hydrophilic sample preparation and allows for m/z calibration in the range between 300 Da and 1 000 Da.

1.1. Storage and shelf life

SpheriCal® is shipped without temperature control. Once received, please store the vials in the freezer at -20°C to ensure a shelf life of 3 years post production. The expiring date is stated on each vial.

Chemically, the SpheriCal® technology can be considered a polyester. As such, the molecules are very stable over a long period of time. However, hydrolysis can occur in aqueous environments at highly acidic or highly basic conditions. Accordingly, we advise you to store aliquots of dissolved SpheriCal® Aqua in the freezer, with a shelf life of 18 months after preparation.

1.2. Order Information

This product (product number PFS-1001) can be re-ordered at https://bit.ly/PFS-1001

2. Instructions for calibration

2.1. Software - Mass Control List

To calibrate your MALDI instrument you will need a Mass Control List. You can either generate your own or, if compatible with your software, use the lists supplied by us on our homepage. You may download a compressed folder with all our products mass control lists at:

https://bit.ly/SpheriCal_MCL

Once downloaded, decompress and place the files the relevant folder on your instrument computer (e.g. *Mass Control Lists* at *D:\Methods\MassControlLists*). Make sure it has the correct file extension (.mcl). If your software requires another file format, you should be able to create your own mass control list with the values provided in Table 1.

You will find "monoisotopic" and "Average" lists. We recommend choosing according to your instrument's resolving power in the intended m/z range and your method (e.g. linear or reflective mode).

Once you have placed the supplied mass list file in the correct folder, or generated your own, the masses will be visible alternatives in your calibrant list in your MALDI acquisition software (e.g.

FlexControl). If this list is not visible, restart your control program and make sure the file has the right file extension and is in the right folder.

The calibration masses below are Na-adducts as these usually are the dominating species for our calibration molecules. For detailed insight on the isotopic pattern, please check the Appedix.

Table 1. Masses and formulas for SpheriCal® Aqua Peptide Low.

Component	Formula	[M+Na] (monoisotopic)	$[M+Na]^+$ (average)
PFS-1022	$C_{12}H_{22}O_8$	317.121 Da	317.288 Da
PFS-2012	$C_{17}H_{30}O_{10}$	417.174 Da	417.404 Da
PFS-1032	$C_{20}H_{36}O_{12}$	491.210 Da	491.482 Da
PFS-1042	$C_{25}H_{44}O_{16}$	623.253 Da	623.607 Da
PFS-1052	$C_{30}H_{52}O_{20}$	755.295 Da	755.722 Da
PFS-1062	$C_{40}H_{70}O_{25}$	973.410 Da	973.969 Da

2.2. Calibration - general

The following is a guide on how to calibrate the MALDI-ToF MS after the previous step is completed. Note that these instructions are made in accordance with Bruker instruments and that you should take care not to deviate from any instrument specific instructions you may have been given.

To ensure the highest possible accuracy, we recommend preparing a calibration spot in direct vicinity to your analyte spots. By generating a pattern as shown in Figure 1, you can use one calibration spot for 8 analyte spots.

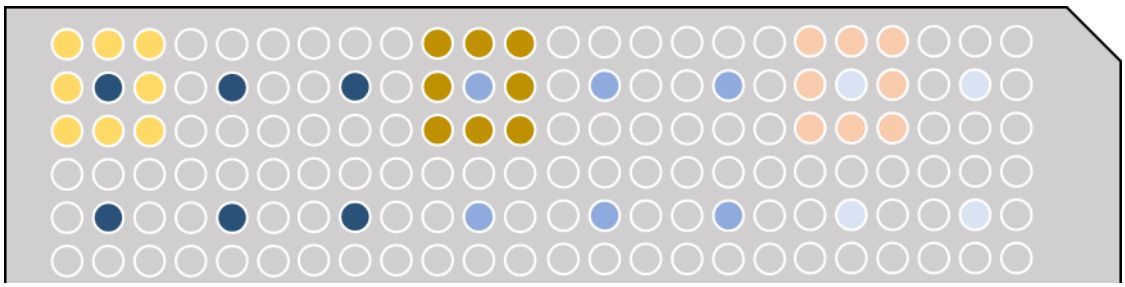

Figure 1. Suggested calibration spot pattern. Blue represents calibration spots. Yellow, brown, and pink represent analyte spots.

2.3. Calibration - preparation of spots by pre-mixing, dried droplet method

For the best results, we suggest using a mixture of 75% acetonitrile-25% water as the solvent. Methanol, ethanol, or THF (and aqueous mixtures thereof) are also suitable. Sodium trifluoroacetate (NaTFA) is recommended as the cation-source, and dihydoxybenzoic acid (DHB) as the matrix. Other suitable matrices for this product are HCCA and graphite.

To achieve calibration spots with good signal intensity and distribution, we recommend to pre-mix calibrant, counter ion, and matrix and store in aliquots.

1) Each new vial contains 50 µg of SpheriCal®. Add 50µL of solvent into the vial to prepare a SpheriCal® solution at a concentration of 1 mg/mL (**solution A**).
2) Prepare a NaTFA solution in the same solvent at a concentration of 2 mg/mL (**solution B**).
3) Prepare a matrix solution in the same solvent at a concentration of 20 mg/mL (**solution C**).
4) Add 5 µL of solution A, 5µL of solution B and 20 µL of solution C to a suitable vial (e.g. an Eppendorf vial or their lid). Mix well to generate **solution D**. This solution contains the calibrant at 0.166 mg/mL and the calibrant:counterion:matrix ratio (by mass) is 1:2:80.
5) Pipette up to 0.5 µL of solution D onto the desired spots on the target plate and leave to dry under gentle air flow.
6) Divide the remaining solution A and D into portions that match your use and store in freezer until needed. Prepared solutions are good to use for up to 18 months. We recommend to use Eppendorf Safe-Lock microcentrifuge tubes. Label with preparation date and content.
7) Once the prepared calibration spots, as well as your analyte spots have dried, you are ready to start your measurements.
8) Measure the MALDI spectrum of a calibration spot, ensure good signal to noise spectra. Compare your collected spectrum with Figure 2. Intensities and intensity ratios of the 6 components will vary, depending on the matrix used.
9) Within the calibration tab of your software, choose the *SpheriCal® Aqua Peptide Low* mass list and click “automatic assignment”. The software should automatically assign the peaks. Click through the assignments and ensure that the software has chosen the correct monoisotopic peaks (cf. Figure 2, bottom). With the available 6 calibration points, you can use advanced algorithms such as *cubic enhanced*. Click *calibrate* to perform your calibration.
10) You are now ready to measure your analyte spots in direct vicinity with a well-calibrated MALDI.

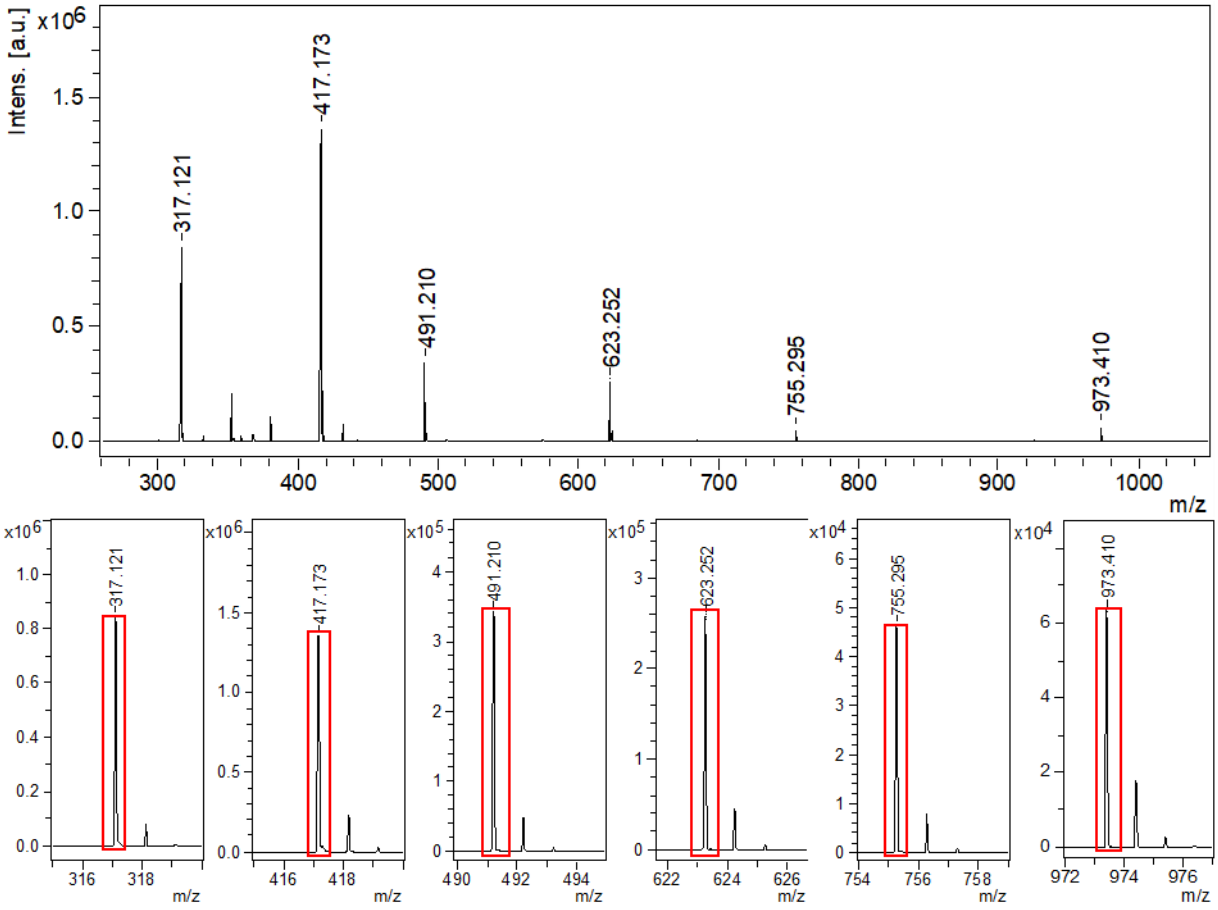

Figure 2. MALDI spectrum of PFS-1001 obtained with graphite as matrix. Monoisotopic peaks highlighted with red boxes.

Notes:

- Changing acquisition parameters will invalidate the calibration. Re-calibrate once you have changed parameters.
- With DHB and HCCA, you will see matrix cluster signals that are not part of the calibration.
- If you find the preparation of solution D troubling, you might consider to purchase our SpheriCal® Mix products, where Polymer Factory has done that job for you. You will find these products here: https://bit.ly/SpheriCal-MIX

Contact

Please feel free to email any inquiries about SpheriCal® at jens.sommertune@polymerfactory.com.

Otherwise, Polymer Factory can be contacted at info@polymerfactory.com.