

Instructions for Use MALDI Biotarget 48



**Disposable MALDI targets for
microorganism identification**

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1 Product Description

The MALDI Biotarget 48 is a disposable MALDI target for microorganism identification in the MALDI Biotyper workflow. It is produced with 48 sample spots and 5 calibrant spots. The single-use design avoids any risk of cross contamination due to inadequate cleaning. Each MALDI Biotarget 48 is also provided with a machine readable unique identification code.

Ordering Information

Product	Part Number
MALDI Biotarget 48 (pack of 25 targets)	# 268711
Biotarget Adapter	# 267615
Barcode Scanner for MALDI Biotarget 48	# 268821
Transport Box for MALDI Biotarget 48 (pack of 10 boxes)	# 270006
MTP MSP Adapter	# 226413
HCCA Matrix, portioned (pack of 10 tubes)	# 255344
Bruker Bacterial Test Standard (pack of 5 tubes)	# 255343

2 Hardware Requirements

microflex™ LT

- + Biotarget Adapter (Part Number # 267615)

autoflex™ speed

- + Biotarget Adapter (Part Number # 267615)
- + MTP MSP Adapter (Part Number # 226413)

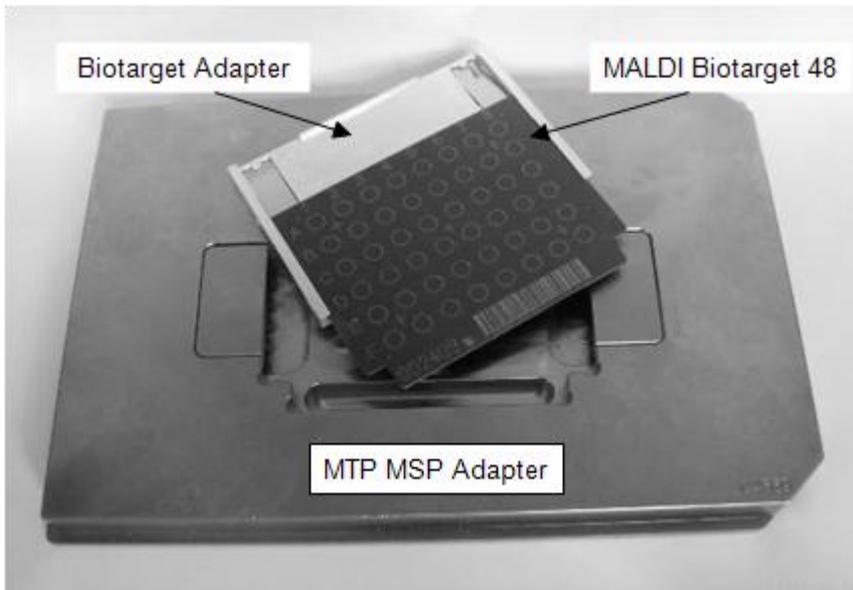


Figure 1 MALDI Biotarget 48 with Biotarget Adapter (Part Number # 267615) and MTP MSP Adapter (Part Number # 226413)

3 Software Requirements

- **MALDI Biotyper 3.0 + Compass 1.3 or later**
- **Biotarget Geometry Setup**
 - Biotarget Geometry Setup can be downloaded from www.bdal.com/268711.
 - Run this setup before using MALDI Biotarget 48 for the first time. The setup enables flexControl to recognize the Biotarget spot geometry.
 - Close the flexControl software before starting the installation.
 - Execute the installation from the User account to be used for the measurement.
- **TargetChangeTool 2.0.1**
 - TargetChangeTool 2.0.1 can be downloaded from www.bdal.com/268711.
 - Unzip this tool and copy it to the desktop of the User account (not the Administrator account).
 - The tool allows preselection of preferred MALDI targets in MALDI Biotyper automation workflows.
 - The Automation Control Wizard must be closed.

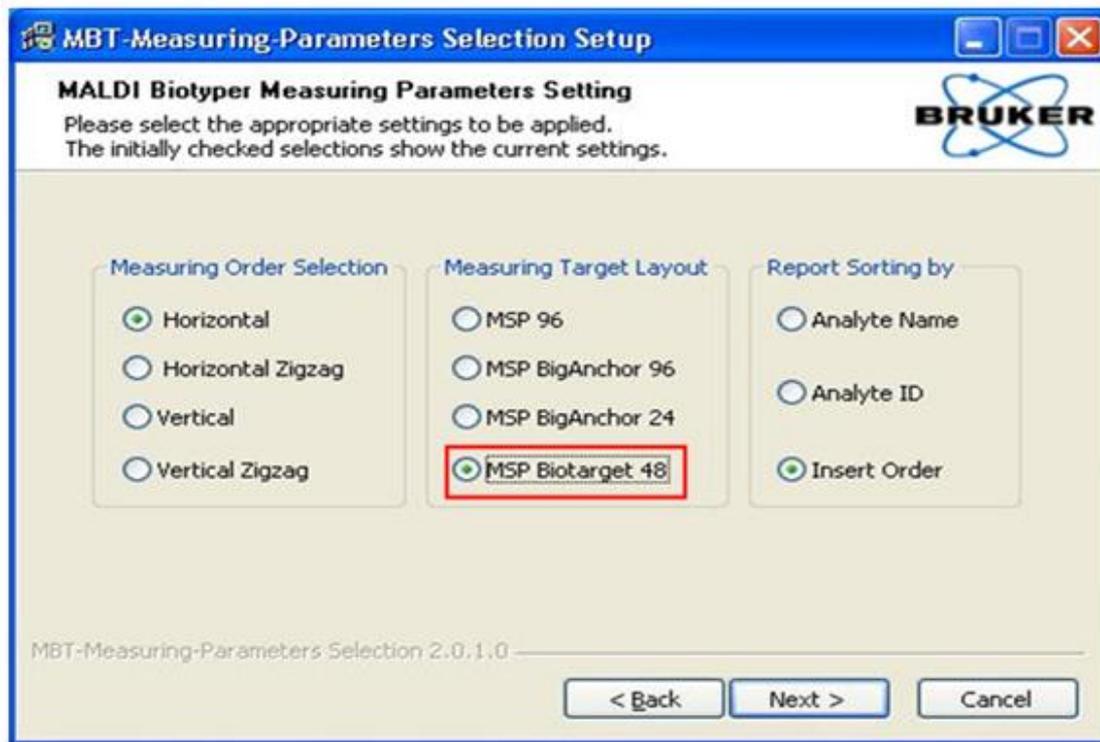


Figure 2 MBT-Measuring Parameters Selection Setup dialog

4 Mounting the MALDI Biotarget 48 on the Biotarget Adapter

Take care not to break the MALDI Biotarget 48 when mounting it on the adapter (see Figure 3).

SHARP HAZARD

The target is fragile and could present a cutting hazard if it is broken or damaged.

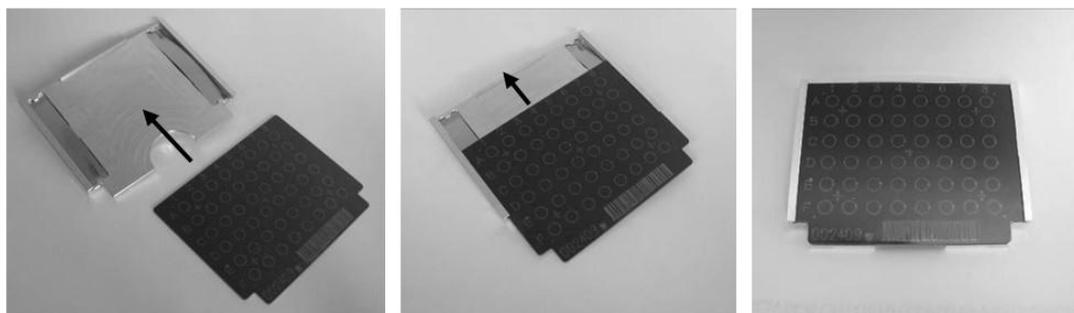


Figure 3 Mounting the MALDI Biotarget 48 on the Biotarget Adapter (Part Number # 267615)

5 Reading the Barcode

Every MALDI Biotarget 48 is equipped with a unique serial number and a machine readable identification barcode, containing the part number, the serial number and two validation digits.

Bruker offers a dedicated CCD barcode reader (Part Number # 268821).

Note Laser scanners cannot read the barcode on the MALDI Biotarget 48.

We recommend that the barcode reader is configured to add a carriage return after the barcode using the barcode in Figure 4.



Figure 4 Configuration barcode. Print out and scan this code to initialize the barcode reader.

6 Loading and Teaching the Target Geometry

The MALDI Biotarget 48 is equipped with five calibrant spots (BTS 0 – BTS 4) labeled with crosses (see Figure 5). The calibration procedures for microorganism identification is described in detail in section 9.

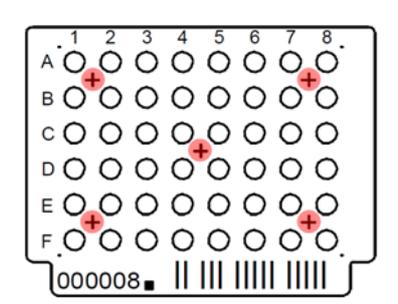


Figure 5 Position of calibrant spots on MALDI Biotarget 48

These spots are also used for geometry teaching.

IMPORTANT The flexControl method must be recalibrated after changing the target geometry to or from the MALDI Biotarget 48 geometry. If autocalibration fails (autocalibration button in flexControl 3.3) the user must calibrate the system manually (see section 9).

To teach the MSP MALDI Biotarget 48 geometry

1. Make sure that **MSP MALDI Biotarget 48** is selected¹ in the **Geometry** drop-down list of flexControl (see Figure 6).

¹If the Geometry cannot be defined (is grayed out), select the **none** option in the **Run** field of the AutoExecute tab.

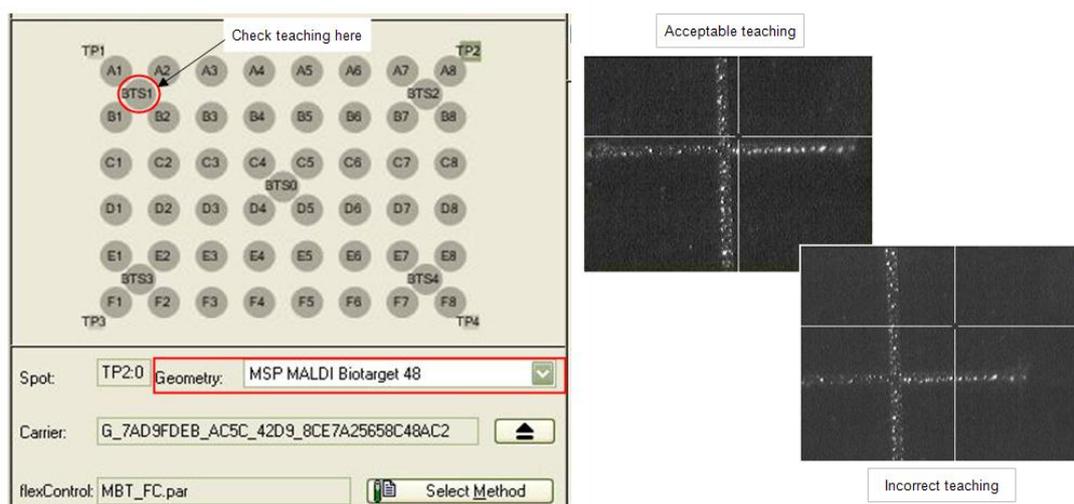


Figure 6 Load the correct target geometry in flexControl and check that the spot teaching is correct.

2. After the MALDI Biotarget 48 has been inserted into the mass spectrometer, always check the teaching by selecting **BTS 1** (see Figure 6) and checking whether the engraved cross on the target surface corresponds with the crosshair in the flexControl video window.
3. If the teaching is incorrect, load the Default Teaching by clicking on the relevant button on the **Sample Carrier** tab in flexControl. Recheck the **BTS 1** spot position.
4. If the teaching is still incorrect, a new teaching must be performed:
 - a. On the **Sample Carrier** tab, select **Advanced > Teach**.
 - b. The Teach Positions window opens. Move the sample carrier to the first teaching position **BTS1** by clicking on the **Go** button next to **BTS1**. Align the position of the cross engraved on the target surface with the crosshair in the flexControl video window. Then click on the **Reached** button next to **BTS1**.
 - c. Teach **BTS2** and **BTS4** in the same way.
 - d. Click **Save As Default Teaching** and **OK**.

7 Microorganism Cultivation (Starting Material)

Cultivation conditions have little effect on spectra generation. Altering growth conditions lead to only minimal variations in the peak lists, with almost all peaks being highly reproducible. Media such as LB, YPD, or blood medium can be used as can different growth states or growth temperatures.

However, in the interests of reproducibility, the same medium and growth conditions should be used for cultivation of microorganisms where possible.

If possible, freshly grown material (grown overnight) should be used. In the case of slow-growing bacteria, cultures grown over several days can be used.

If cultivation plates are stored at 4°C the quality of spectra decreases relatively quickly (within a few days). Plates can be stored at room temperature for several days.

8 Sample Preparation on MALDI Biotarget 48

There are two standard sample preparation protocols for microorganism profiling with MALDI Biotyper and the MALDI Biotarget 48:

- Direct Transfer Procedure (see section 8.2)
- Ethanol / Formic Acid Extraction Procedure (see section 8.3)

Both protocols are applicable to steel targets as well as to the MALDI Biotarget 48.

In addition, there is a protocol for matrix preparation (see section 8.1) and a protocol for Bruker Bacterial Test Standard (referred to as 'Bruker BTS') sample preparation (see section 8.4).

8.1 Matrix Preparation Procedure

Chemicals Required

- HCCA matrix, portioned (Bruker Part Number # 255344)
- Standard solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%) should be purchased from Sigma-Aldrich (# 19182). **Note:** For in-house preparation, only use chemicals of the highest purity available (e.g. CHROMASOLV HPLC or LC-MS solvents).



Standard solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%), is classified as a hazardous chemical: DANGER (H: 225, 302, 315, 319; P: 210, 305+351+338)

Preparation of Matrix Solution

1. Dissolve the HCCA matrix, portioned in 250 μ l standard solvent (concentration: 10 mg HCCA/ml) by vortexing at room temperature until the solution is clear (all crystals have to be in solution).

The dissolved matrix solution can be stored in the dark (UV sensitive) at room temperature for several days.

8.2 Direct Transfer Sample Preparation Procedure

Sample Preparation using Direct Transfer Procedure:

1. Smear biological material (single colony) as a thin film directly onto a MALDI Biotarget 48 sample spot.

Pipette tips or inoculation loops can be used. Only a small (barely visible) amount of material is required for measurement.

2. Overlay the biological material with 1 μ L HCCA matrix solution.
3. Allow the sample spot to air dry before analysis.

8.3 Ethanol/Formic Acid Extraction Sample Preparation Procedure

If the direct transfer method fails, this method can be used to increase the efficiency of protein extraction and provide improved results.

To prevent any oxidation reactions which lead to peak shifting it is very important to work rapidly. Sample spots should be overlaid with HCCA matrix solution as quickly as possible after drying (within a few minutes).

Chemicals and Materials Required

- Ethanol absolute, ≥99.8% (GC), (undenaturated) (# 24102, Sigma-Aldrich GmbH)
- 70% Formic acid (puriss. p.a., ~98% (T) [# 06440, Sigma-Aldrich GmbH])
- Ultra pure water, CHROMASOLV® for HPLC (# 270733, Sigma-Aldrich GmbH)
- Acetonitrile ≥99.9%, LC-MS CHROMASOLV® grade at Fluka (# 34967, Sigma-Aldrich GmbH)
- Eppendorf tubes (e.g. article no. 0030 120.086) and tips. **Note:** Always use genuine Eppendorf tubes and tips; plasticware from other manufacturers may release plasticizers that will adversely affect the results.

Sample Preparation using Ethanol/Formic Acid Extraction Procedure:

1. Pipet 300 µL water into a clean Eppendorf tube.
2. Transfer up to 10 mg of biological material into the tube and mix thoroughly to suspend the cell effectively in the water.

The amount of material used can vary from a single colony up to 10 mg.

3. Add 900 µl 100% ethanol to the tube and mix thoroughly.
4. Centrifuge at 13,000 rpm in a desktop centrifuge for 2 minutes and decant the supernatant. Centrifuge for a further 2 minutes and remove residual ethanol from the pellet using a pipet.

For microorganisms present at low concentrations or those with a robust cell wall, dry the EtOH pellet for some minutes at room temperature to increase the extraction efficiency.

5. Add between 1–80 µL 70% formic acid to the pellet and mix thoroughly by pipetting and / or by vortexing. Add an equal volume of acetonitrile to the tube and mix carefully.

The volume of formic acid and acetonitrile added to the pellet should be proportional to the amount of biological material transferred in step 2.

	Small single colony	Large single colony	1 µL inoculation loop	10 µL inoculation loop
Formic acid	1–5 µL	5–15 µL	10–40 µL	30–80 µL
Acetonitrile	1–5 µL	5–15 µL	10–40 µL	30–80 µL

6. Centrifuge at 13,000 rpm in a desktop centrifuge for 2 minutes and pipet 1 µL supernatant onto a MALDI Biotarget 48 sample spot.
7. As soon as the sample spot has dried, overlay the sample with 1 µL HCCA matrix solution.
8. Allow the sample spot to air dry before analysis.

8.4 Bruker BTS Sample Preparation Procedure

Chemicals and Materials Required

- Bruker Bacterial Test Standard (Bruker Part Number # 255343)
- Standard solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%) should be purchased from Sigma-Aldrich (# 19182). **Note:** For in-house preparation, only use chemicals of the highest purity available (e.g. CHROMASOLV HPLC or LC-MS solvents).



Standard solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%), is classified as a hazardous chemical: DANGER (H: 225, 302, 315, 319; P: 210, 305+351+338)

- Screw cap micro tubes (Sarstedt, # 72.730.003) with screw caps (Sarstedt, # 65.716.002).

Solubilization and Preparation of Bruker BTS Aliquots

1. Add 50 μL of standard solvent to the Bruker BTS pellet and dissolve by pipetting up and down at least 20 times at room temperature.

Avoid foaming of the solution!

2. Incubate the solution for 5 minutes at room temperature and pipette up and down at least 20 times.
3. Centrifuge at 13,000 rpm for 2 minutes at room temperature.
4. Pipette 5 μL aliquots of the supernatant into screw cap micro tubes.
5. Store the aliquots at -18°C or below.

The dissolved Bruker BTS can be stored for up to 5 months at -18°C or below. A few oxidation products may be detected, which do not significantly influence spectra quality.

Preparation of Bruker BTS Sample

Note Frozen Bruker BTS aliquots should be thawed at room temperature and mixed by flicking the screw cap micro tube with a finger. Screw cap micro tubes should be closed and stored at -18°C or below immediately after preparation of Bruker BTS sample spots. Each 5 μL aliquot contains enough material for at least eight sample spots on the MALDI Biotarget 48.

1. Deposit 0.5 μL of Bruker BTS solution on any of the BTS positions on the target.
2. Dry the Bruker BTS sample at room temperature.
3. Overlay the Bruker BTS sample spot with 0.5 μL HCCA matrix solution immediately after drying.
4. Dry the sample at room temperature.

9 Calibration of Bruker MALDI-TOF Mass Spectrometer Using Bruker Bacterial Test Standard

9.1 Calibration Points Used

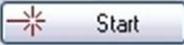
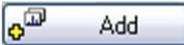
Table 1 Calibration points with a mass tolerance limit of ± 300 ppm

Protein	Reference mass (average mass)	± 300 ppm range
RL29 [M+2H] ²⁺	3637.8 Da	3636.7 Da – 3638.8 Da
RS32 [M+H] ⁺	5096.8 Da	5095.3 Da – 5098.3 Da
RS34 [M+H] ⁺	5381.4 Da	5379.8 Da – 5383.0 Da
RS33meth [M+H] ⁺	6255.4 Da	6253.5 Da – 6257.3 Da
RL29 [M+H] ⁺	7274.5 Da	7272.3 Da – 7276.7 Da
RS19 [M+H] ⁺	10,300.1 Da	10,297.0 Da – 10,303.2 Da
RNAse A [M+H] ⁺	13,683.2 Da	13,679.1 Da – 13,687.3 Da
Myoglobin [M+H] ⁺	16,952.3 Da	16,947.2 Da – 16,957.4 Da

9.2 Calibration Procedures

Both manual and automatic calibration procedures are available. The manual procedure can be used with all versions of flexControl whereas for the automatic procedure flexControl 3.3 or later is required.

9.2.1 Manual Calibration Procedure

1. Insert the MALDI target prepared with the Bruker BTS sample into the MALDI-TOF mass spectrometer.
2. Ensure that flexControl is running.
3. Use the MBT_FC.par standard flexControl method (Linear and Positive mode) for measurement and calibration.
4. Manually measure a sum spectrum by summing 6 x 40 laser shots from different raster positions on the Bruker BTS spot. On each desired raster position, click the **Start** button  and then the **Add** button  to record a total of 240 laser shots, displayed as **Added: 240**. The laser energy level should be optimized.

Optional: A convenient alternative to the manual measurement of a sum spectrum is to:

- 1 click the **AutoXecute** tab
- 1 select the autoX method MBT_AutoX from the **Method** drop-down list (Figure 7)
- 1 click the **Run method on current spot**  button.



Figure 7 AutoXecute tab with selection of the **MBT_AutoX** method for automatic sum spectrum measurement

This automatically measures a sum spectrum.

5. Click the **Single Buffer** toolbar button  to hide the currently displayed single spectrum. This enables the **Sum Buffer** toolbar button  and displays the measured sum spectrum.
6. Click the **Smooth Spectrum** toolbar button  to smooth the sum spectrum (Figure 8).

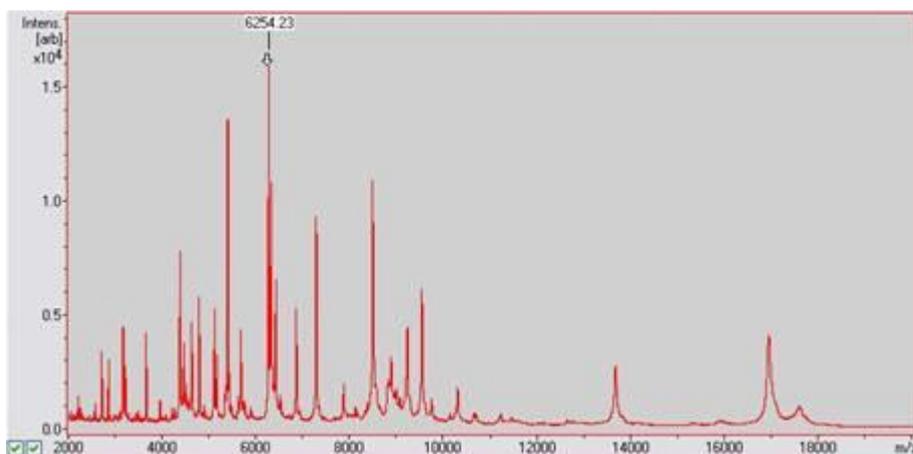
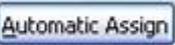


Figure 8 Smoothed sum spectrum of Bruker BTS

7. Click the **Calibration** tab and select the calibration file **MBT_Standard** from the **MassControl List** drop-down list (Figure 9).



Figure 9 Calibration tab showing the Mass Control List

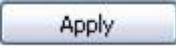
8. Click the **Automatic Assign** button  to automatically select the correct calibration peaks.
9. Check if the automatic assignment of calibration peaks worked properly. The calibration peak list should show that all peaks are assigned and that the maximum deviation **Err/ppm** is not more than ± 300 ppm for any of the peaks (Figure 10).

Name	Ref. Mass/Da	Cur. Mass/Da	Err/ppm	Err/Da
✓ RL29 [M+2H] ²⁺	3637.80000	3637.00930	-217.36	-0.79070
✓ R532 [M+H] ⁺	5096.80000	5097.16292	71.21	0.36292
✓ R534 [M+H] ⁺	5381.40000	5381.93407	99.24	0.53407
✓ R533meth [M+...]	6255.40000	6256.11042	113.57	0.71042
✓ RL29 [M+H] ⁺	7274.50000	7274.85374	48.63	0.35374
✓ R519 [M+H] ⁺	10300.10000	10299.41065	-66.93	-0.68935
✓ RNAse A [M+H] ⁺	13683.20000	13681.01687	-159.55	-2.18313
✓ Myoglobin [M+...]	16952.30000	16954.00226	100.41	1.70226

Figure 10 Result of automatic assignment of calibration peaks where all peaks are assigned and the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks

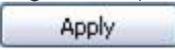
Depending on the result, proceed as follows:

- If all peaks are assigned and the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks, automatic assignment of calibration peaks was successful. Proceed to step (12).
- If all peaks are assigned but the maximum deviation **Err/ppm** is more than ± 300 ppm for any of the peaks, repeat steps (4) to (9).
- If a few peaks are assigned and the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks, proceed to step (10).
- If a few peaks are assigned but the maximum deviation **Err/ppm** is more than ± 300 ppm for any of the peaks, repeat steps (4) to (9).
- If none of the peaks is assigned, proceed to step (11).

10. Click the **Apply** button  and repeat automatic peak assignment by clicking the **Automatic Assign** button .

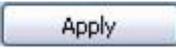
Check if all peaks are assigned and if the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks.

- If all peaks are assigned and the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks, automatic assignment of calibration peaks was successful. Proceed to step (12).
- If not all of the peaks are assigned or if the maximum deviation **Err/ppm** is more than ± 300 ppm for any of the peaks, repeat steps (4) to (9).

11. Manually select the peak at m/z 6255.4 Da by clicking left hand side of the peak in the spectrum. The vertical line next to the peak must turn from red to green for successful peak assignment (the corresponding data is entered in the calibration peak list). Click the **Apply** button  and repeat automatic peak assignment by clicking the **Automatic Assign** button .

Check if all peaks are assigned and if the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks.

- If all peaks are assigned and the maximum deviation **Err/ppm** is less than ± 300 ppm for all of the peaks, automatic assignment of calibration peaks was successful. Proceed to step (12).
- If not all of the peaks are assigned or if the maximum deviation **Err/ppm** is more than ± 300 ppm for any of the peaks, repeat steps (4) to (9).

12. After all peaks have been automatically assigned with a maximum deviation **Err/ppm** of less than ± 300 ppm for all of the peaks, click the **Apply** button .

- Save the new calibration values in the flexControl method (MBT_FC.par). Clicking the **Save method as** toolbar button  opens the **Save flexControl Method As** dialog. Click the **Save** button. Confirm the replacement of the file (MBT_FC.par) by clicking the **Yes** button in the confirmation dialog (Figure 11).



Figure 11 Confirmation dialog on replacing the specified method file

9.2.2 Automatic Calibration Procedure

Note flexControl 3.3 or later is required for the automatic calibration procedure.

- Insert the MALDI target prepared with the Bruker BTS sample into the MALDI-TOF mass spectrometer.
- Ensure that flexControl is running.
- Use the MBT_FC.par standard flexControl method (Linear and Positive mode) for measurement and calibration.
- Select **Tools > Method Calibration Parameters** (Figure 12) to set or check the calibration settings of the flexControl method.

Note Setting of calibration parameters is only required for the first (initial) calibration. For later calibrations, only the selection of the correct autoX method (MBT_AutoX) must be checked.

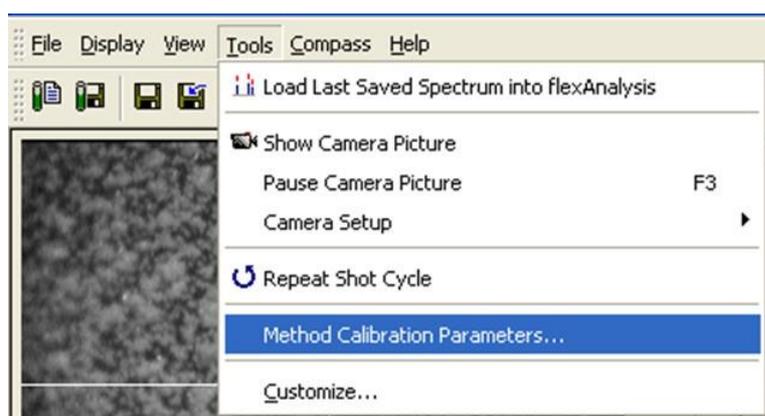


Figure 12 Method Calibration Parameters command from Tools menu

- Enter or check the auto calibration parameter settings shown in Figure 13. Then click the **OK** button.

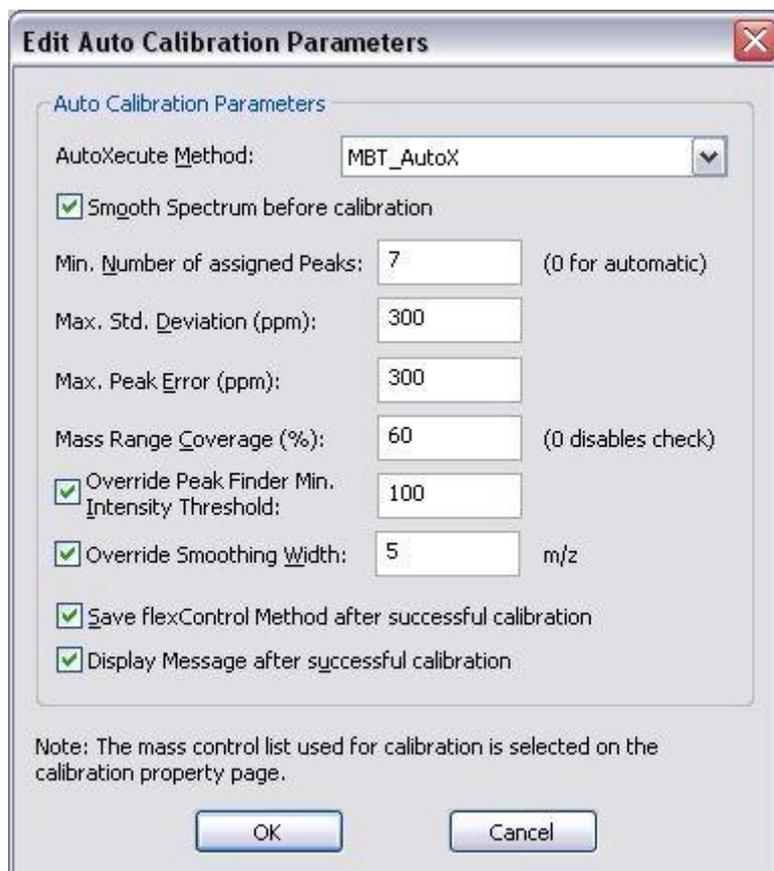


Figure 13 Edit Auto Calibration Parameters dialog

6. On the **Calibration** tab (Figure 9), check that the **Peak Assignment Tolerance** parameter is set to the maximum value of 1000 ppm (Figure 14).

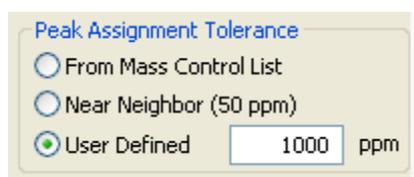


Figure 14 Peak Assignment Tolerance parameter

7. To start the calibration procedure, click the **Calibrate** button  **Calibrate**.

A sum spectrum is automatically acquired by summing the 6 x 40 laser shots from different positions on the Bruker BTS spot and calibration is automatically completed. A calibration information dialog opens after successful calibration (Figure 15). Click the **OK** button to complete calibration.

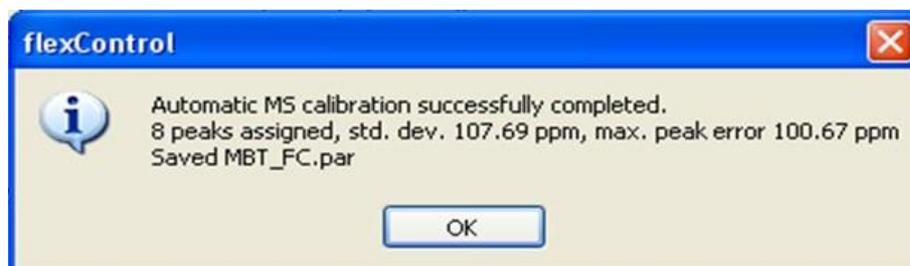


Figure 15 Calibration Information dialog

8. If the calibration has failed, repeat the automatic calibration procedure, steps 4 to 7, or follow the manual calibration procedure described in section 9.2.1.

10 Support

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