

Instructions for Use

Bruker Bacterial Test Standard

Mass calibration standard showing a typical *Escherichia coli* DH5 alpha peptide and protein profile plus additional proteins.

For use in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

CARE products are designed to support our worldwide customers with high-quality consumables, accessories and dedicated kits.

The CARE product range is specifically optimized and certified for use with all Bruker Daltonics systems.

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1 Product Description

Bruker Bacterial Test Standard (referred to as 'Bruker BTS') contains a carefully manufactured extract of *Escherichia coli* DH5 alpha that shows a characteristic peptide and protein profile in MALDI-TOF mass spectra. The extract is spiked with two additional proteins that extend the upper boundary of the mass range covered by Bruker BTS. The overall mass range covered by Bruker BTS is 3.6 to 17 kDa.

Each tube contains enough material for the preparation of around 40 MALDI target plate spots.

This kit is for research use only. It is not for use in diagnostic procedures.

Ordering Information

Product	Part Number
Bruker Bacterial Test Standard, 5 tubes	# 255343

2 Inspection, Storage and Stability

2.1 Inspection on Arrival

Check the Bruker BTS package on arrival. If it is damaged, check the tubes. If the tubes are damaged, the Bruker BTS must not be used. Dispose of the Bruker BTS (tubes and package) following the guidelines outlined in the Material Safety Data Sheet for the product and contact Bruker Daltonik GmbH (care@bdal.de) for a replacement.

2.2 Storage on Arrival

	-18°C	Bruker BTS is shipped at ambient temperature but must be stored at -18°C or below immediately on arrival. The expiry date on the package is valid for the enclosed Bruker BTS when stored at -18°C or below on arrival.
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2.3 Storage after Aliquoting

Bruker BTS must be solubilized before use. We strongly recommend that dissolved Bruker BTS is aliquoted and frozen using 0.5 mL screw cap micro tubes (for instance, Sarstedt, # 72.730.003) with screw caps (Sarstedt, # 65.716.002).

	-18°C	Frozen, dissolved Bruker BTS can be stored for up to 5 months at -18°C or below. With time, oxidation products may be detected. These oxidation products do not significantly influence spectra quality.
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3 Risk and Safety Information

This product is not classified according to Regulation (EC) No. 1272/2008 and is therefore not classified according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). In the manufacturer's experience, the product has no harmful effect when used and handled according to specifications. However, all materials may present unknown hazards and should be handled with caution. For more information, read the Material Safety Data Sheet available for download at www.bdal.com/msds.

Additional chemicals may be required for procedures described in these Instructions for Use. Carefully read the Material Safety Data Sheet provided by the supplier and follow general safety regulations when handling chemicals or biohazardous material.

4 Software Requirements

flexControl versions 3.0 and earlier require a free software patch to support Bruker BTS. Please contact your local Bruker Daltonics Service Team for installation support.

5 Bruker BTS Sample Preparation Procedure

Preliminary Remarks

Poor sample preparation will degrade sensitivity and will yield low resolution and poor reproducibility. The generation of ions through MALDI depends on the presence of an optimal ratio of matrix substance to analyte. For best results, use freshly prepared solutions and chemicals of the highest purity available (e.g. CHROMASOLV LC-MS solvents).

Chemicals and Materials Required

- Bruker IVD Matrix HCCA, portioned (# 290200) or Bruker Matrix HCCA, portioned (# 255344) (referred to as Bruker (IVD) HCCA, HCCA = α -cyano-4-hydroxycinnamic acid), which must be prepared as described in the relevant Instructions for Use.
- Standard solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%) from Sigma-Aldrich (# 19182), which has been tested by Bruker Daltonik GmbH and is recommended for solubilization of Bruker BTS.
- Screw cap micro tubes (Sarstedt, # 72.730.003) with screw caps (Sarstedt, # 65.716.002).
- MALDI target of the type selected for your workflow.

Equipment and Tools Required

Centrifuge, pipettes, pipette tips

5.1 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 then mix by pipetting 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 and close tubes tightly.
5. Store the aliquots at -18°C or below.

5.2 Preparation of Bruker BTS Sample on a MALDI Target

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 four sample spots.

1. Deposit 1 μL of Bruker BTS solution on any position of the MALDI target.
2. Dry the Bruker BTS sample at room temperature.
3. Overlay the Bruker BTS sample spot with 1 μL Bruker (IVD) HCCA solution immediately after drying.
4. Dry the sample at room temperature.

A homogeneous preparation should be observed.

6 Calibration of Bruker MALDI-TOF Mass Spectrometer Using Bruker BTS

6.1 Calibration Points Used

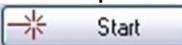
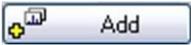
Table 1 Calibration points with a mass tolerance limit of ± 300 ppm

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] ⁺	10300.1 Da	10297.0 Da – 10303.2 Da
RNAse A [M+H] ⁺	13683.2 Da	13679.1 Da – 13687.3 Da
Myoglobin [M+H] ⁺	16952.3 Da	16947.2 Da – 16957.4 Da

6.2 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.

6.2.1 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 standard flexControl method MBT_FC.par (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: Alternatively, a sum spectrum can be measured automatically by:

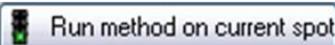
1. click the **AutoXecute** tab,
1. select the autoX method MBT_AutoX from the **Method** drop-down list (Figure 1),
1. click the **Run method on current spot**  button.



Figure 1 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 2).

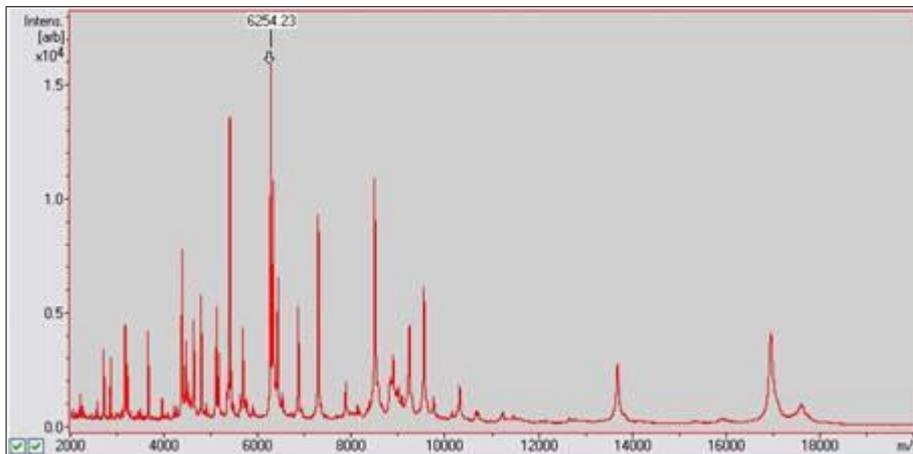


Figure 2 Smoothed sum spectrum of Bruker BTS

7. Click the **Calibration** tab and select the calibration file **MBT_Standard** from the **MassControl List** drop-down list (Figure 3).



Figure 3 Calibration tab showing the Mass Control List

8. Click the **Automatic Assign** button  to automatically pick 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 4).

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 4 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:

- 1 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).
- 1 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).
- 1 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).
- 1 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).
- 1 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.

- 1 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).
- 1 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).

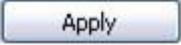
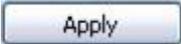
11. Manually select the peak at m/z 6255.4 Da by clicking left hand side of the peak in the spectrum. The vertical line next to the peak must turn from red to green for successful peak assignment (the corresponding data is entered in the calibration peak list). Click the **Apply** button  and repeat 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.
- 1 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).
 - 1 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 .
13. 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 5).



Figure 5 Confirmation dialog on replacing the specified method file

6.2.2 Automatic Calibration Procedure

Note flexControl 3.3 or later is required for the automatic 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 standard flexControl method MBT_FC.par (Linear and Positive mode) for measurement and calibration.

4. Select **Tools > Method Calibration Parameters** (Figure 6) 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.

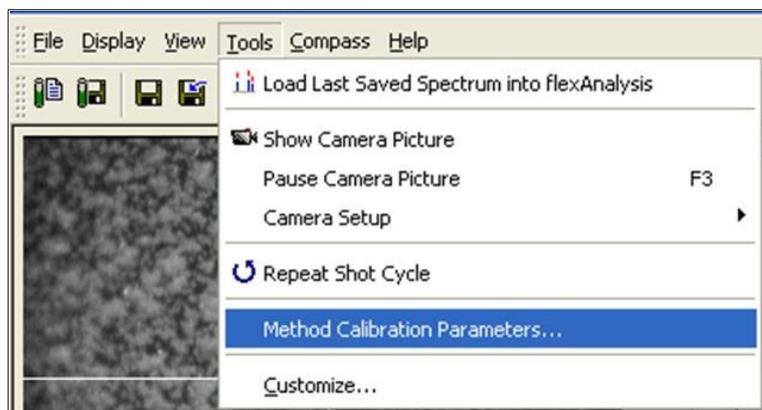


Figure 6 Method Calibration Parameters command from Tools menu

5. Enter or check the auto calibration parameter settings shown in Figure 7. Then click the **OK** button.

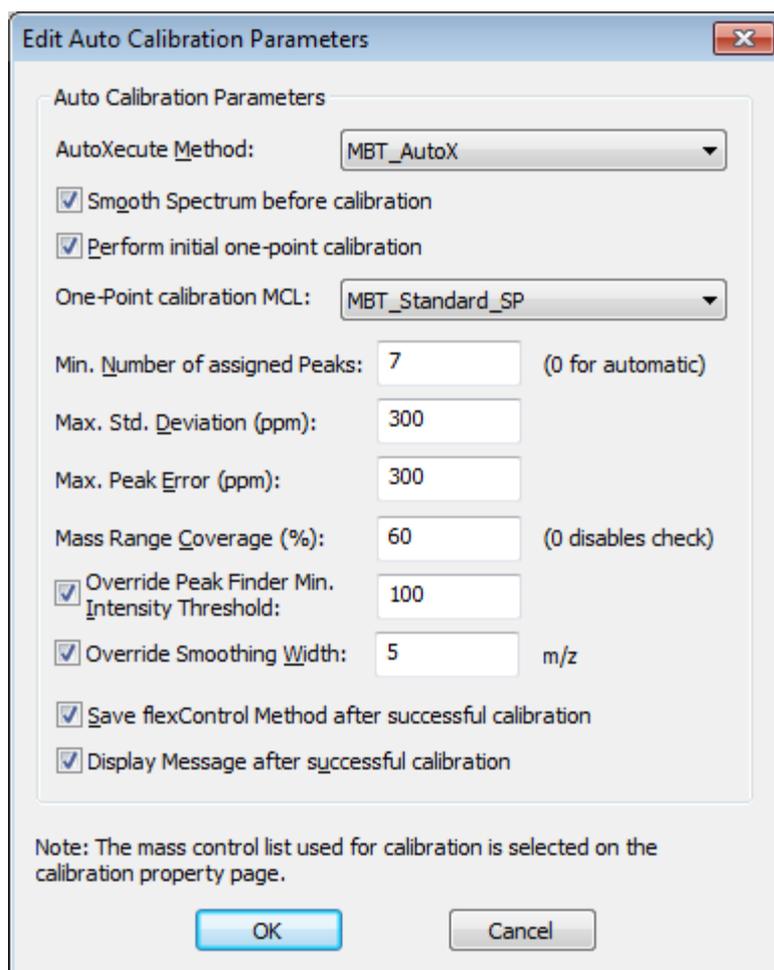


Figure 7 Edit Auto Calibration Parameters dialog

6. On the **Calibration** tab (Figure 3), check that the **Peak Assignment Tolerance** parameter is set to the maximum value of 1000 ppm (Figure 8).

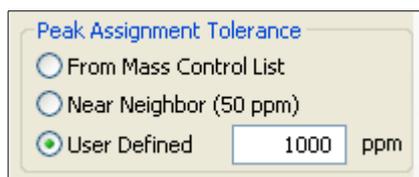
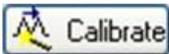


Figure 8 Peak Assignment Tolerance parameter

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

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 9). Click the **OK** button to complete calibration.

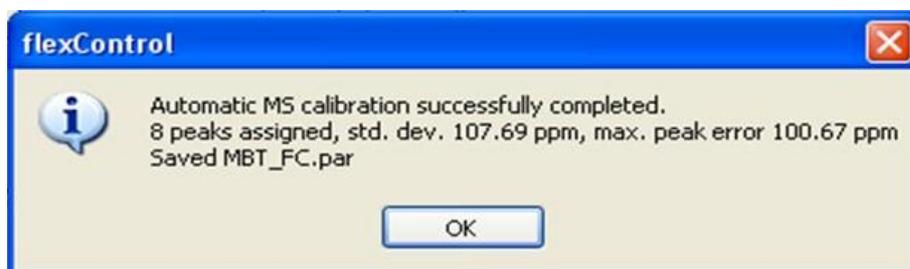


Figure 9 Calibration Information dialog

8. If the calibration has failed, repeat the automatic calibration procedure, steps (4) to (7), or follow the manual calibration procedure described in section 6.2.1.

7 Manufacturer



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