FlashNote

Did you know MALDI can identify your protein in 15 min?

Rapid protein digest methods combined with MALDI-TOF sample analysis and a dedicated software workflow can accelerate the determination of a protein fingerprint against a reference profile. MALDI Rapid Identity Testing is a highly selective assay that can be used to confirm the sequence of a recombinant protein to support research and development but can also be validated as a identity method for product release.

Challenge

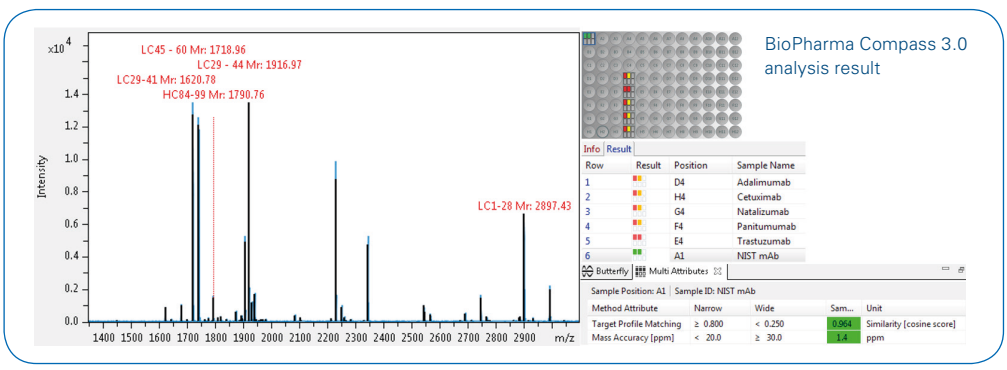
During the development of a monoclonal antibody and all the way through the commercialization of the molecule, it is necessary to have a robust identity assay. Especially if the molecule is processed in a facility that handles multiple drug substance/products. Identity can be determined based on binding affinity or biophysical properties like pI. However in some instances those approaches do not provide sufficient selectivity. Peptide mapping is a way to address this problem at the expense of increased assay complexity.

Solution

Using MALDI Rapid Identity Testing it is possible to confirm the identity of purified proteins such as monoclonal antibody identities in less than 15 min sample preparation and 10 seconds per MS measurement. The measurement produces a unique singly charged spectrum as a easily interpreted fit for purpose result. Automated software processing in BioPharma Compass 3.0 reduces the operator learning curve and simplifies the implementation of GLP guidelines.

Start saving time!

MALDI Rapid Identity Testing provides an easy to learn and robust assay to assess the identity of recombinant proteins.



BioPharma Compass 3.0 analysis result

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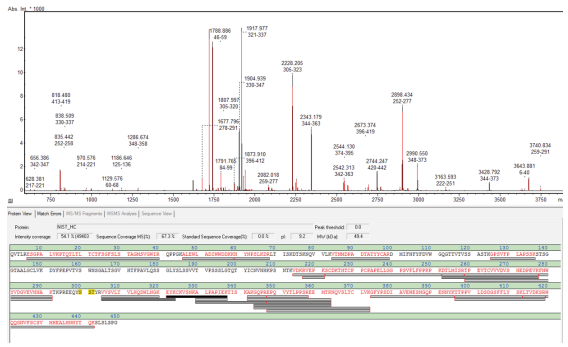
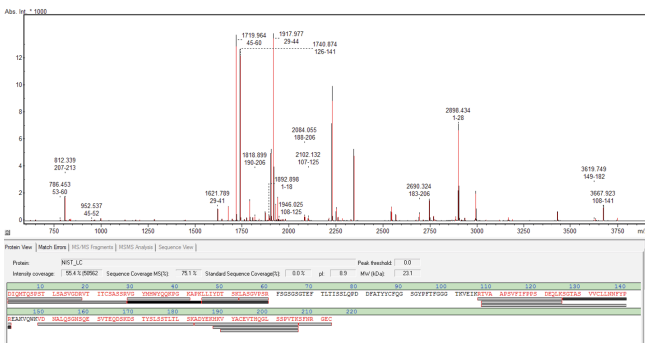
NISTmAb Heavy Chain



NISTmAb Light Chain



1 Materials

- Dithiothreitol (DTT) 50 mM in water
- Trifluoroethanol (TFE)
- 25 mM ammonium bicarbonate (NH_4HCO_3, ABC) in water with pH ~8.5
- 100 mM ammonium phosphate monobasic ($(NH_4)H_2PO_4$) in water (AP100)
- 0.1% and 10% trifluoroacetic acid (TFA) in water
- Solvent mixture TA85 (85:15 (v/v) acetonitrile: TFA 0.1% in water)
- 10 mg/mL MALDI matrix HCCA (Bruker # 8201344) in acetone
- Recrystallization solution composed of 784µL TA85, 8 µL 10% TFA and 8 µL AP100
- 100ng/µL trypsin (Promega #V5111) prepared in ABC buffer
- 10 mg/ml NIST mAb, humanized IgG1k monoclonal antibody (RM8671) in its formulation buffer
- Bruker MTP BigAnchorChip sample plate (#8280788)

2 Method

Denaturation

Add 0.25 µL of DTT (50 mM) and 1.25 µL of TFE into 1µL NIST mAb solution, incubate for 5 min at 50°C.

Digestion

Add 15.5 µL ABC buffer (25 mM), then add 4 µL of trypsin solution, incubate for 5 min at 50°C.

Thin layer preparation

Fill a 10 µl pipette tip with 10 µl of HCCA matrix solution (10g/L in acetone) and drag the pipette slowly over the AnchorChip spots to yield homogeneous thin layers of HCCA matrix.

Spotting

Dilute and acidify the digested mAb sample by adding 22 µl of 0.1 % TFA (final mAb digest concentration of 1.5 pmol/µL). Add 1 µL of 2%TFA to the spot before spotting the digest to help preserve the thin layer.

Spot 1 µL acidified digest on a HCCA thin layer spot and incubate at room temperature for ~3 min (do not let dry).

Once incubation is complete, add 5 µl 0.1% TFA to the spotted digest aliquot and remove the whole droplet from the plate. Repeat wash procedure by adding and removing immediately 5 µL of 0.1 % TFA.

Recrystallization

Add 1µl of recrystallization solution, let dry under room conditions.

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