FlashNote

Rapid MALDI-MS assay for mAb development-Clone Selection

During biopharma development such as clone selection and fast analysis return times are required to accelerate decision making and reduce costs. Rapid protein digestion with integrated MALDI-TOF sample analysis is used in clone selection workflows to screen glycan profiles in intact Fc domains. A Protocol was developed to achieve analysis return time from intact antibody sample to Fc-glycoprofiling within 30 min.

Challenge

Therapeutic monoclonal antibodies (mAb) are typically manufactured using cultured mammalian cells. The integrity and quality of mAbs need to be assessed during clone selection and later during upstream processing. Clone selection may easily involve 10 s-100 s of clones and screening for suitable candidates can be a lengthy step in the manufacture of the therapeutic mAb products.

Solution

A simple and rapid screening method may save a lot of time and resources in the development process. Here a MALDI-TOF MS based LC-free method is presented to measure the glycoform distribution of the NISTmAb Fc/2 fragments right after a simple IdeS digestion. Multiple attributes such as the match of the glycan profile with a reference profile are reported in BioPharma Compass 3.0. It provides multiple data points for decision making of which clones to select for further rounds of screening. The 2+ charge state species are used for analysis.

Eureka!

MALDI rapid clone selection assay provides an integrated and robust way to select your clone.

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Materials

- 10 mg/mL NISTmAb, humanized IgG1k monoclonal antibody (RM8671) in its formulation buffer
- Bruker MTP BigAnchorChip sample plate (#8280788)
- 2,5-Dihydroxyacetophenone (2,5-DHAP) matrix (Bruker #8231829)
- FabRICATOR® enzyme (IdeS), 8×100 Units (Genovis #A0-FR1-008)
- 0.1% and 2% trifluoroacetic acid (TFA) in water
- 18 mg/mL diammonium hydrogen citrate (DAC)

Method

Intact protein dilution

For intact NISTmAb measurement, dilute 10 mg/mL NISTmAb at least 1:20 using 0.1% TFA to decrease salt concentration.

Digestion

FabRICATOR® (IdeS) is a cysteine protease that digests IgG at one single amino acid position below the hinge. Add 10 µL NISTmAb (10mg/mL) to the IdeS enzyme tube containing 100 units of lyophilized FabRICATOR® and incubate for 30 min at 37°C.

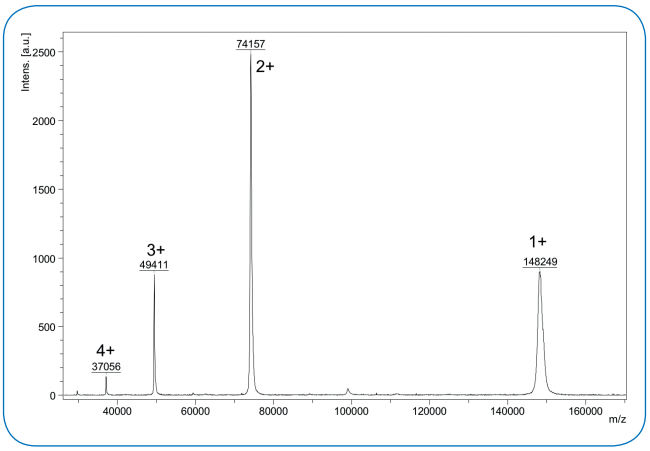
Dilute an aliquot of the digest at least 1 to 20 with 0.1% TFA.

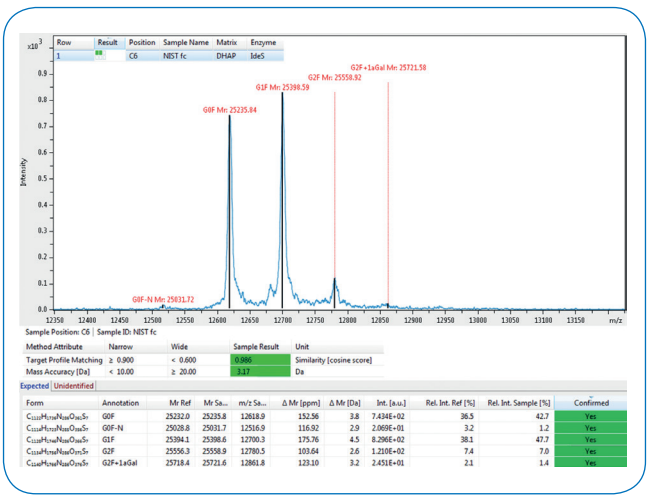
DHAP Matrix preparation

Dissolve 7.6 mg 2,5-DHAP in 375 µL EtOH. Add 125 µL 18 mg/mL DAC. Mix 2 µL of the sample solution with 2 µL of the 2% TFA solution. Add 2 µL of the matrix solution. Pipette up and down this ternary mixture until the crystallization starts. Spot 0.5 µL of the crystal suspension onto the target.

Spotting

Prepare the diluted intact NISTmAb and digest aliquot following the DHAP preparation protocol.





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