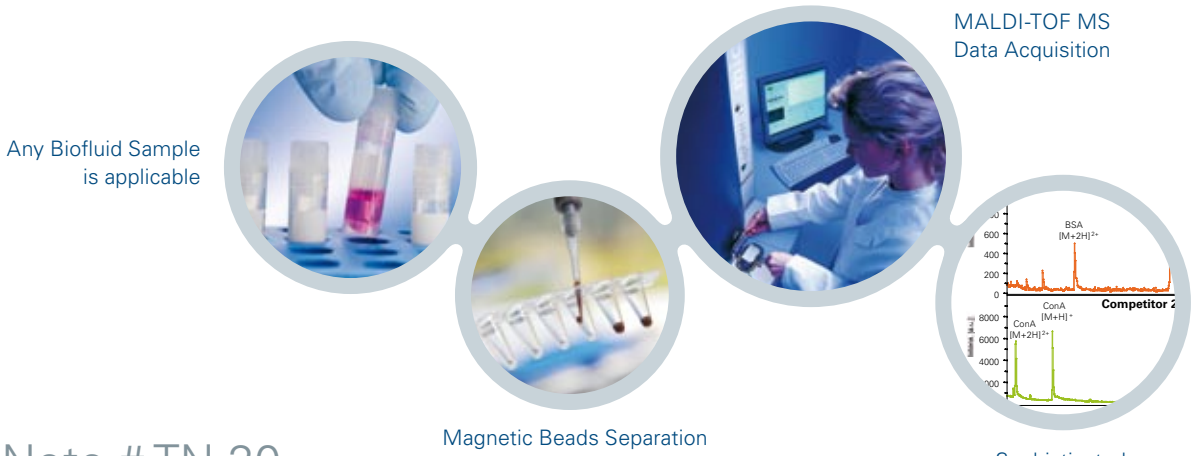
Bruker Daltonics



Technical Note # TN-20

CLINPROT Glycobeads: Verification of Long-Term Stability and Batch-to-Batch Reproducibility

Bruker Daltonics meets the growing demands of glycomic analysis with a unique combination of CLINPROT™ glyco-bead separation technology, superior performance MALDI-TOF/TOF mass spectrometers and advanced ESI-MS/MS systems supported by sophisticated bioinformatic software. With the Bruker Daltonics mass spectrometry workflow, glycoproteins from complex samples like human serum can be reproducibly captured and identified within a dynamic range of eight orders of magnitudes. Besides glycoprotein identification and characterization as well as glyco-structure predictions, CLINPROT glycobeads are suitable for sample fractionation prior to profiling analyses.

Bruker Daltonics offers a well-established portfolio of CLINPROT glycobeads that are functionalized with either lectins like concanavalin A (Con A), wheat germ agglutinin (WGA), Lens Culinaris Agglutinin (LCA), artocarpus integrifolia (AIA or Jacalin) or boronic acid (BA) (Fig. 1). These ready-to-use glyco-kits enhance Bruker Daltonics' separation technology in the field of glycoprotein analysis and clinical proteomics research. With a combination of different optimized beads, intelligent enrichment strategies for glycosylated peptides and proteins prior to mass spectrometry enable advanced glycomic analyses.

Superior quality assurance procedures

Outstanding glycomic studies strongly depend on reliable and reproducible technologies. Especially, the long-term stability and batch-to-batch reproducibility of the applied sample preparation products within a current study are absolutely mandatory. Therefore, Bruker Daltonics performs thoroughly quality controls of the CLINPROT glycobeads in order to provide high end sample preparation products.

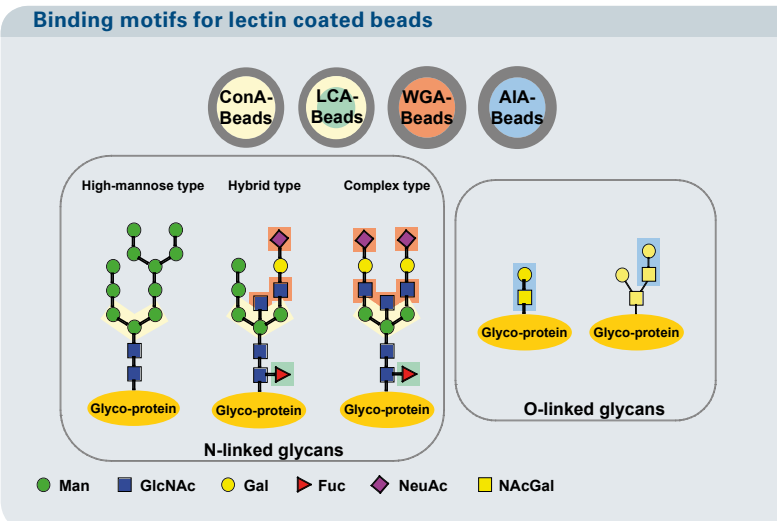


Fig. 1: Binding motifs of different lectin-coated glyco-capturing beads

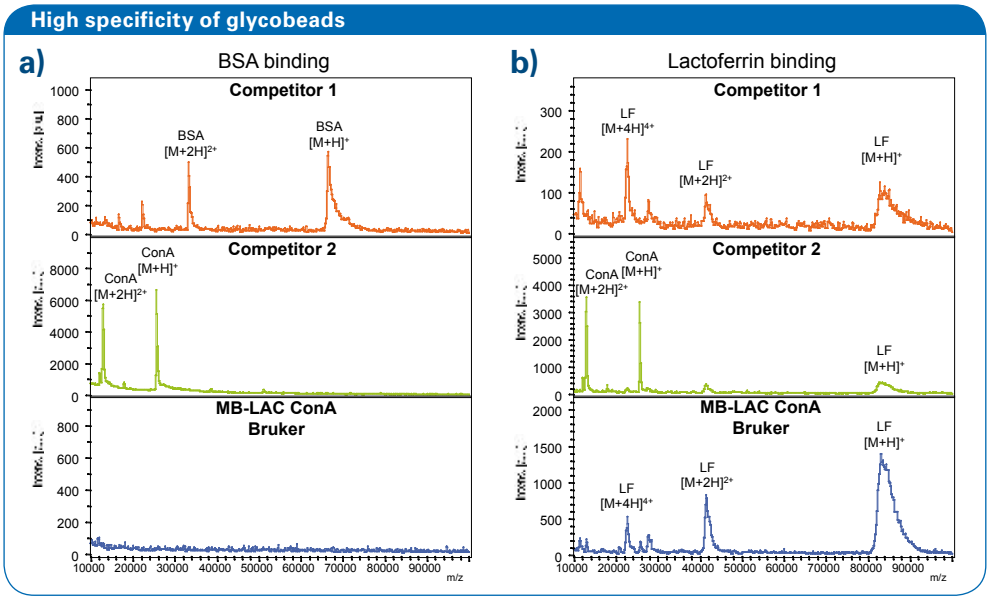


Fig. 2: Comparison of the binding of BSA (a) and Lactoferrin (b) by means of different glyco-capturing particles derived from different suppliers. The binding of model proteins was performed according to the product information. Spectra were acquired on an autoflex II TOF/TOF in the linear mode. 300 shots were acquired.

The stability and functionality of magnetic particles which have been covalently modified with proteins depend on the stability of the corresponding proteins. In order to guarantee specific enrichment features within a given shelf life period, the performance of the respective beads has to be carefully controlled. Compared to beads which are functionalized with chemical groups like boronic acids, ion exchanger groups or alkanes, protein modified beads comprise an unequally higher risk to lose functionality due to proteins' half life.

Figure 2 shows the high specificity of Bruker Daltonics' glycobeads. To ensure that this outstanding performance is also assured on the customer site even after months and unfavourable environments, both the shelf life and stability during shipment of the respective glycobeads are thoroughly investigated. The quality assurance described in the following is consequently applied to every batch of beads produced.

Glycobeads functionality tests

An important measure of the functionality of the magnetic glyco-capturing particles is the binding specificity, e.g. the specific binding in relation to the unspecific binding. Denaturation of the covalently attached lectins, i.e. ConA, WGA and LCA would result in a decrease of the specific interaction of glyco-structure and lectin and an increased enrichment of non-glycosylated proteins due to hydrophobic interactions. For the three different covalently coupled lectins, Lactoferrin (LF) was used as a positive control and the non-glycosylated bovine serum albumin (BSA) was used as negative control. Lactoferrin was choosen as a positive control since it comprises several different glycosylation sites providing the required binding motifs for the different beads. The specificity of the beads as function of time was analyzed by employing either mass spectrometry or the determination of the specific binding capacity.

Mass spectrometric performance as a function of time

For different testing time points the mass spectra of the model proteins BSA, LF and RNase B were acquired and evaluated with respect to the ratio of unbound and bound proteins. In the case of BSA, no peak was detected in the eluate for all beads. The same applied to RNase B in the case of the glycobeads LCA and WGA beads because the glycoprotein RNase B comprises a single high mannose type glycan which does not present the required binding motifs for LCA and WGA. In the opposite, Lactoferrin was bound by all three lectins since it comprises different glycosylation sites and glycan types. The ratio of bound to unbound protein varied for the different glycobead types but was stable over the investigated time period. Figure 3 exemplarily shows the MS spectra after capturing of BSA (a), LF (b) and RNase B (c) directly after synthesis of ConA beads and 20 months later. The ratios between bound and non-bound material were almost the same, demonstrating that the beads have not lost their outstanding performance during the storage time.

Determination of the binding capacity as a function of time

The specific binding capacity of the lectin coated beads after storage at 4°C (40°F) was determined by a BCA assay (Pierce) as the amount of bound LF or BSA, respectively. Since the employed lectins consist of different non-covalently attached subunits, a bleeding of the ConA beads during elution might happen. Thus, it was necessary to consider this by running an empty buffer control and correct

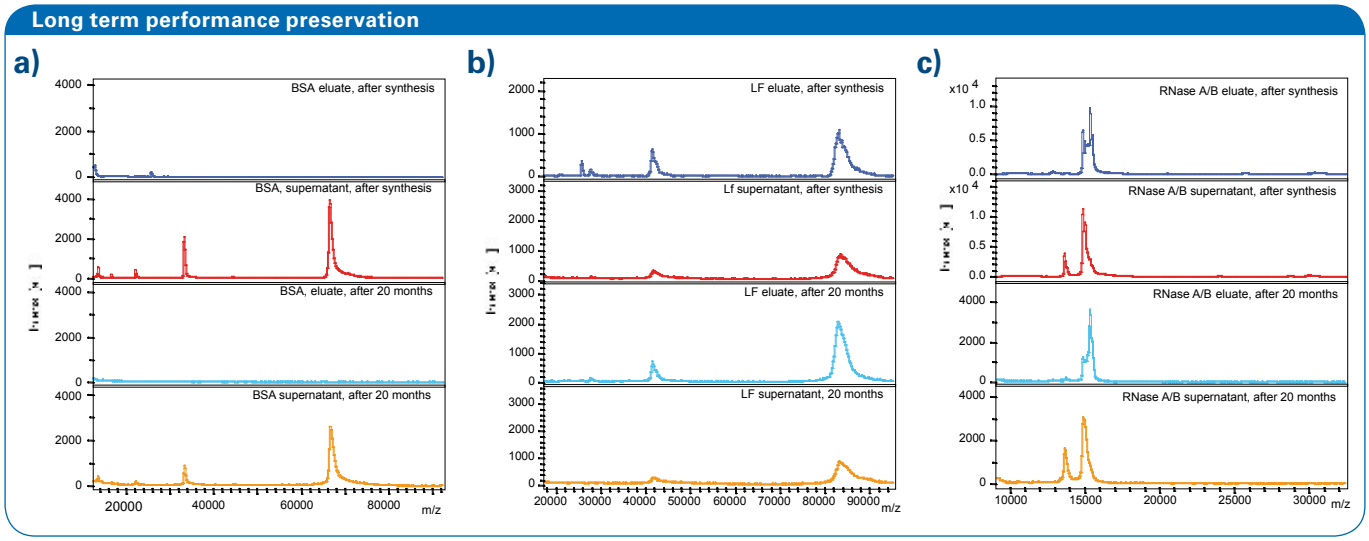


Fig. 3: Mass spectra of the supernatants and the eluates of BSA (a), LF (b) and RNase B (c) after binding to ConA beads performed directly after synthesis and after 20 months of storage. The binding of model proteins was performed according to the product information. Spectra were acquired on an autoflex II TOF/TOF in the linear mode. 300 shots were acquired.

the specific and non-specific binding, accordingly. For each type of the respective beads, corresponding thresholds had been defined characterizing the limits for non-specific and specific binding. Figure 4a shows the binding capacities of Con A beads over a time period of 20 months. After this storage time no loss of functionality could be observed as well as no increased non-specific binding was detected. The defined thresholds for Lactoferin (at least 20µg per mg glycobeads) and for BSA and buffer (less than 6µg per mg beads, respectively) were not exceeded.

The activity of ConA beads has been monitored for about 12 months. During this time the defined quality criteria were fulfilled (Fig 4b). The threshold for specific binding was > 12 µg LF per mg beads and for non-specific capturing < 2 µg BSA per mg beads (after buffer correction, respectively). Similar results were obtained for WGA beads. The performance of the beads was nearly constant over a time period of 12 months (Fig. 4c). All measuring points for the binding of LF were above 20 µg per mg beads and the values for the non-specific and the buffer control were below 6 µg per mg beads.

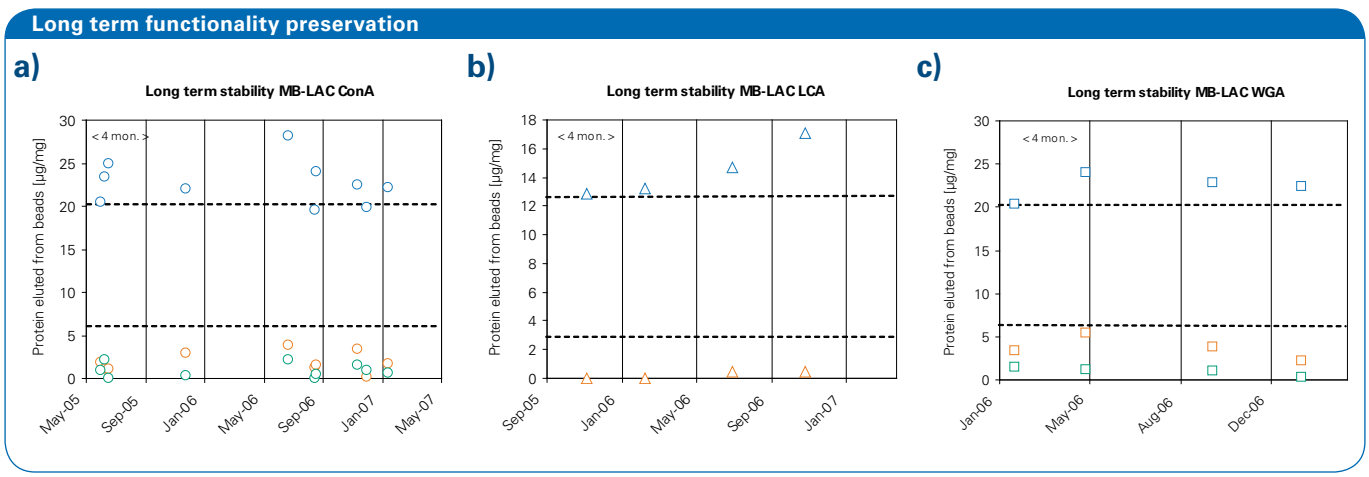


Fig. 4: Time course of the specific binding capacity of different glyco-capturing magnetic particles determined as the amount of LF or BSA, respectively, which was detected after binding of saturating amounts of proteins and subsequent elution. Detailed protocols available from the author.

Simulating shipping conditions

A further critical parameter for the performance of the beads is the storage temperature. In particular, when the beads are shipped the recommended temperature of 4°C (40°F) might be difficult to keep. To evaluate the influence of temperature variations on the bead functionality, beads were stored at different temperature conditions for a time period of 10 days. Subsequently, the binding capacities were determined. Fig.5 shows the results of the above described quality assurance procedure after storage of the ConA, LCA and WGA glycobeads for 10 days at 4°C (40°F), room temperature (RT) and 30°C (86°F), respectively. The analysis of the binding capacities revealed nearly no loss of activity. Even at the increased temperature of 30°C (86°F) the beads did not lose their functionality.

Conclusions

Bruker Daltonics ready-to-use glycobead kits ensure sophisticated glycoprotein analysis and clinical proteomics research. Performing shelf life analysis and shipment simulations, Bruker Daltonics certifies the quality of the respective products on the customer site, thereby minimizing the risk of sample loss. The investigations of stability of the different glycobeads revealed a constant performance of the beads during the analysis periods.

Specificity tests and binding capacity tests are performed with each manufactured bead and kit batch. Therefore, customers always get the most superior and reproducible beads for their sophisticated research.

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Glyco Kit MB-LAC WGA
Glyco Kit MB-LAC Con A
Glyco Kit MB-LAC Jacalin

Kits available from www.care-bdal.com

No influence of storage temperature on functionality

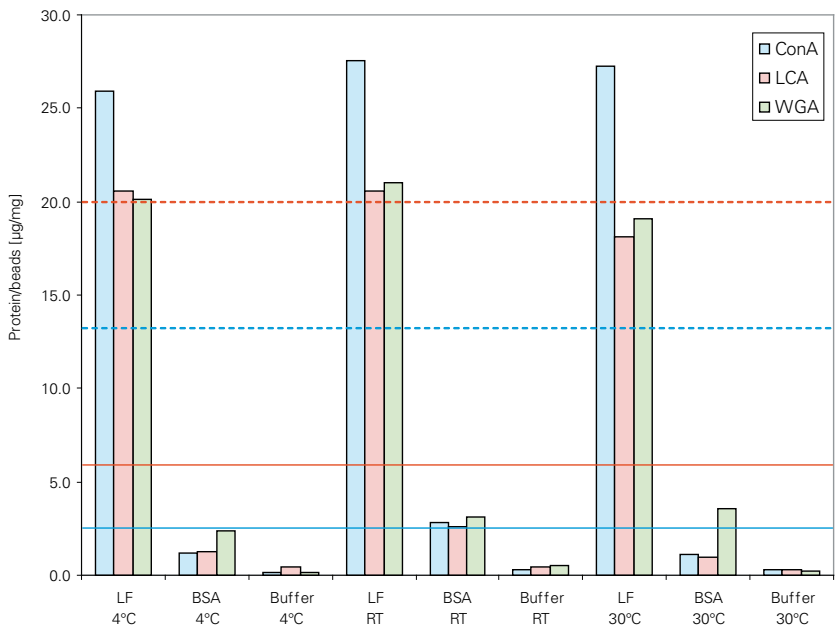

Fig. 5: Binding capacities of ConA, LCA and WGA beads for LF, BSA and buffer after storage for 10 days at 4°C, RT or 30°C, respectively. The thresholds for the minimum binding of Lactoferrin by ConA and WGA beads (red dashed line) and by LCA beads (blue dashed line) and the maximum binding for BSA or the buffer for ConA and WGA beads (red line) and for LCA beads (blue line) are earmarked in the diagram. WGA beads scarcely fall below the lower threshold for the binding of Lactoferrin after incubation at 30 °C.

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