Deglycosylation studies on Maurice, Wes, LabChip GXII Touch HT, and SDS-PAGE

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The method of origin SDS-PAGE is increasingly replaced by CE-SDS automated platforms as Maurice by ProteinSimple, Wes by ProteinSimple, and LabChip GXII Touch HT by PerkinElmer. In the last study the influence of various sample preparation conditions (denaturation temperature, reducing agents) as well as the usage of various molecular weight (MW) markers on the MW determination was investigated. Furthermore, precision and repeatability studies are performed.

Varying the sample preparation conditions result in a maximum 10 % deviation of the calculated MW to the reference MW based on amino acid sequences of aglycosylated proteins, while the glycosylated proteins α-2-Macroglobulin and the heavy chain of Matuzumab, an IgG antibody, show deviations up to 45 % on the investigated approaches.

To evaluate the influence of the glycosylation pattern on the MW determination by SDS-PAGE and CE-SDS, two deglycosylation studies protocols (PNGase F (Promega) and Protein Deglycosylation Mix II (New England Biolabs, NEB)) were performed. The PNGase F protocol of Promega was performed to break the N-linked carbohydrates of Ovalbumin, α-2-Macroglobulin, and Matuzumab. Furthermore, N-Cadherin, SynCam 1, Erythropoetin (EPO) and CD74 were added in a second protocol to also investigate proteins with larger carbohydrate chains and more complex quarternary structures. To additionally break O-linked carbohydrates, the Deglycosylation (Enzyme) Mix 2 of New England Biolabs was utilized.