



S-BIO Glycan Analysis Services for Bioprocessing

High-throughput, Quantitative Glycan Analysis

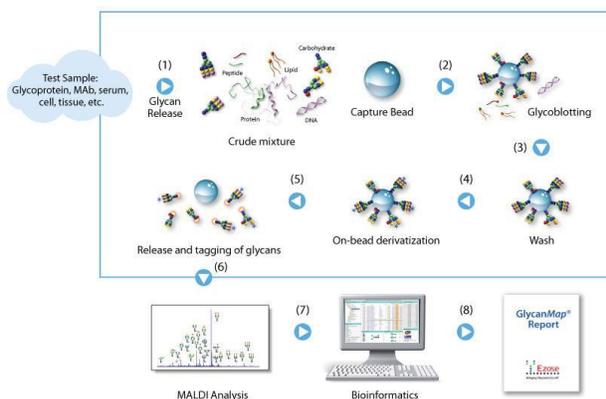
S-BIO specializes in the rapid and efficient analysis of either *N*-linked or *O*-linked glycans present on antibodies and other glycoproteins. Our proprietary GlycanMap® platform combines a bead-based glycan purification in a fully automated sample processing system with high-throughput MALDI-TOF mass spectrometry to generate rapid, reproducible glycan profiles. The resulting qualitative and quantitative information can be employed to achieve a variety of endpoints related to cell line selection, fermentation and process development, formulation stability and product characterization.

S-BIO's GlycanMap® Assay Services provide:

- Fully automated, 96-well format sample processing to maximize throughput and repeatability
- Simultaneous quantitation of both neutral and acidic *N*-linked oligosaccharides, including sialylated glycans
- Absolute quantitation of *N*-linked glycans, including low abundance species
- Compatibility with antibodies and complex glycoproteins of varying levels of purity
- A new solution to accelerate your early biotherapeutic development programs by enabling rapid glycoform analysis across literally hundreds of samples
- Multiple service options to meet your glycan analysis needs, including our *NEW* GlycanMap® Xpress™ assay, designed for rapid *N*-glycan screening of large sample sets during cell line selection and upstream process development

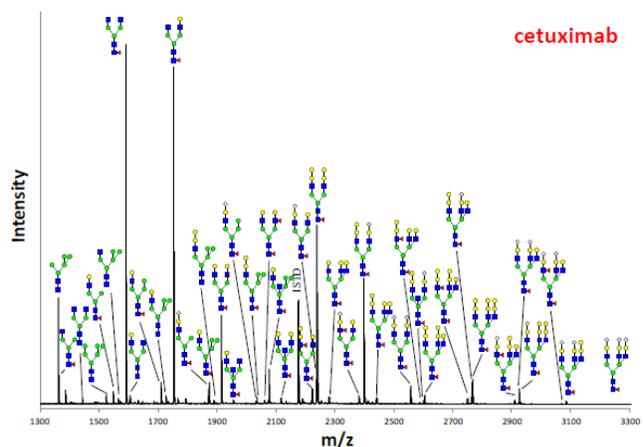
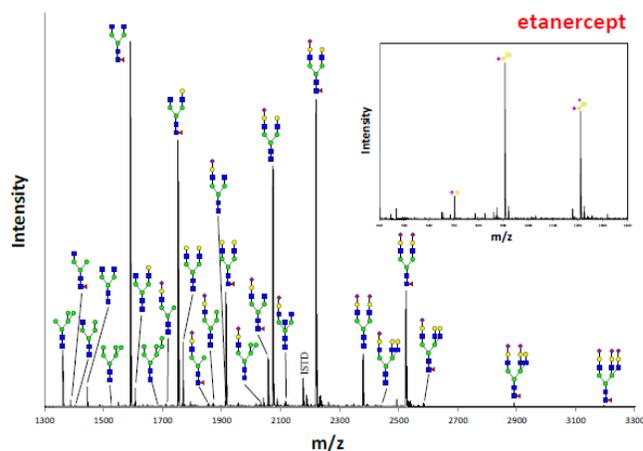
GlycanMap® Platform

S-BIO's proprietary GlycanMap® assay relies on robust enrichment of glycans on a solid phase, followed by quantitative MALDI-TOF mass spectrometry and custom bioinformatics. After transferring samples to 96-well plate and adding internal standard, proteins are digested with trypsin and glycans released with PNGase F (1). Glycans are captured on beads using a reversible covalent linkage, a process referred to as glycoblotting (2). After on-bead washing (3), sialic acids are stabilized by methylesterification (4). Glycans are released from the beads and the reducing ends tagged (5). Released glycans are analyzed by MALDI-TOF mass spectrometry in the positive, reflector mode (6). Proprietary bioinformatics programs determine glycan compositions and quantities (7) and generate a report (8).



Case Study: GlycanMap® Analysis of Biotherapeutics

Due to the influence of glycans on the mechanism of action, clearance rate, immunogenicity and efficacy of monoclonal antibodies and other glycoprotein therapeutics, detailed characterization of these molecules remains an important challenge in both the development and production of biotherapeutics. Glycans in an Fc fusion protein (etanercept) and a monoclonal antibody (cetuximab) were analyzed using the GlycanMap® assay. A total of 26 *N*-glycans were detected in etanercept, with concentrations ranging from 23 to 7967 pmol/mg protein. Three *O*-linked glycans were also detected. In cetuximab, the *N*-glycans detected included all 21 glycans previously reported, plus an additional 22 glycans and included glycans containing two fucoses, *N*-glycolylneuraminic acid and/or terminal α -1,3-galactosyl residues.



N-linked Glycans Detected in Etanercept			
m/z	Composition (Hex HexNAc Fuc Neu5Ac Neu5S2)	Proposed Structures	Concentration (pmol/mg)
1362.53	5 2 0 0 0		881
1387.57	3 3 1 0 0		96
1403.55	4 3 0 0 0		24
1444.59	3 4 0 0 0		313
1524.58	6 2 0 0 0		24
1590.65	3 4 1 0 0		7131
1606.65	4 4 0 0 0		243
1686.66	7 2 0 0 0		23
1708.68	4 3 0 1 0		31
1752.72	4 4 1 0 0		5561
1768.72	5 4 0 0 0		1230
1854.75	4 3 1 1 0		60
1870.74	5 3 0 1 0		76
1911.76	4 4 0 1 0		102
1914.77	5 4 1 0 0		2545
2032.80	6 3 0 1 0		56
2057.82	4 4 1 1 0		1158
2073.83	5 4 0 1 0		6017
2114.81	4 5 0 1 0		69
2219.90	5 4 1 1 0		7967
2378.92	5 4 0 2 0		1336
2438.93	6 5 0 1 0		27
2524.98	5 4 1 2 0		3044
2585.01	6 5 1 1 0		95
2890.10	6 5 1 2 0		98
3195.21	6 5 1 3 0		23

About S-BIO

S-BIO is dedicated to advancing glycomics to enable new innovations in disease diagnosis and therapy. S-BIO's glycan analysis services are targeted to audiences who find glycan analysis a bottleneck in their development efforts and would benefit from our high-throughput capabilities to provide rapid and reliable glycan analysis. The company tailors these capabilities to the needs of our partners, typically under fee-for-service agreements. Established in 2009 as a US company, S-BIO is wholly-owned by Shionogi & Co., Ltd., Osaka, Japan. Since initiating operations S-BIO has established over thirty external projects and partnerships with pharma, biotech and academic collaborators. These include publicly announced collaborations or presentations with Genentech, Hirosaki University, Kyowa Hakko Kirin, Merck, NUI Galway, Sigma-Tau, and Fast Forward (National Multiple Sclerosis Society).

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