SIGMA-ALDRICH®
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Product Information

SILu™Lite SigmaMAb
Universal Monoclonal Antibody Standard, human
recombinant, expressed in CHO cells

Catalog Number **MSQC4**
Storage Temperature –20 °C

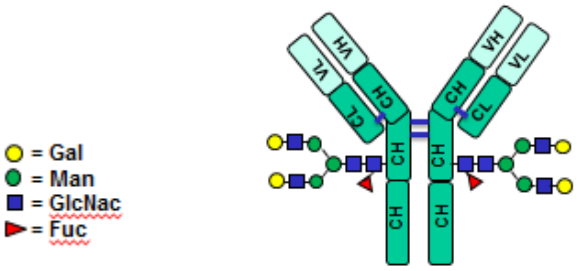
Product Description

SILu™Lite SigmaMAb is a recombinant human monoclonal antibody with a molecular mass of ~150 kDa expressed in CHO cells. It is designed for optimization of accurate intact mass analysis of monoclonal antibodies, biosimilars, and pharmaceutical products. Accurate intact mass analysis of such large biomolecules can provide comprehensive information about structural and post-translational modifications such as glycosylation. Other information such as heterogeneity, batch-to-batch variation, amino acid truncation, and N-terminal Lys processing, aggregation, and degradation can be determined. Intact mass analysis is also very important for formulation and storage in therapeutic monoclonal antibody drug development.

SigmaMAb is an IgG1 antibody with a lambda light chain. It consists of two identical heavy chains and two identical light chains. The heavy chains and light chains are linked by one disulfide bond. The heavy chains are linked by two disulfide bonds located in a hinge domain. The other 12 cysteine bonds are intramolecularly restricted to six different globular domains (Figure 2).

The antibody sequence has been evaluated by intact mass and peptide mapping using four different enzymes: chymotrypsin, Asp-N and Glu-C endoproteinases, and trypsin. Sequence coverage of 100% was obtained.

Structural Information



Sequence Information

1. SigmaMAb Heavy Chain:
   EVQLVESGGGLVQPGGSLRLSCVASGFTLNNYD
   MHWVRQGIGKGLEWVSKIGTAGDRYYAGSVKGR
   FTISRENAKDSLYLQMNSLRVGDAAVYYCARGAG
   RWAPLGAFDIWGQGTMVTVSSASTKGPSVFPLA
   PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
   LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
   TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
   APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
   VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
   TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
   EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
   CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
   DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
   NHYTQKSLSLSPG

2. SigmaMAb Light Chain:
   QSALTQPRSVSGSPGQSVTISCTGTSSDIGGYNF
   VSWYQQHPGKAPKLMIYDATKRPSGVPDRFSGS
   KSGNTASLTISGLQAEDEADYYCCSYAGDYTPGV
   VFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK
   ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT
   PSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVT
   HEGSTVEKTVAPTECS

Component

Each vial of SigmaMAb contains 1 mg of antibody lyophilized from a solution of phosphate buffered saline (PBS). Vial content is determined by measuring A_{280} and using an extinction coefficient ($E^{0.1\%}$) = 1.4.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
SigmaMAb recovery is maximized when phosphate buffer, pH 6**–**7, is used to reconstitute the lyophilized product.
Note: **Avoid PBS** for reconstitution.

Reconstitute the contents of the vial by adding 500 µL of ultrapure water or phosphate buffer, and mixing vigorously. The solubilized product can be further diluted as needed.

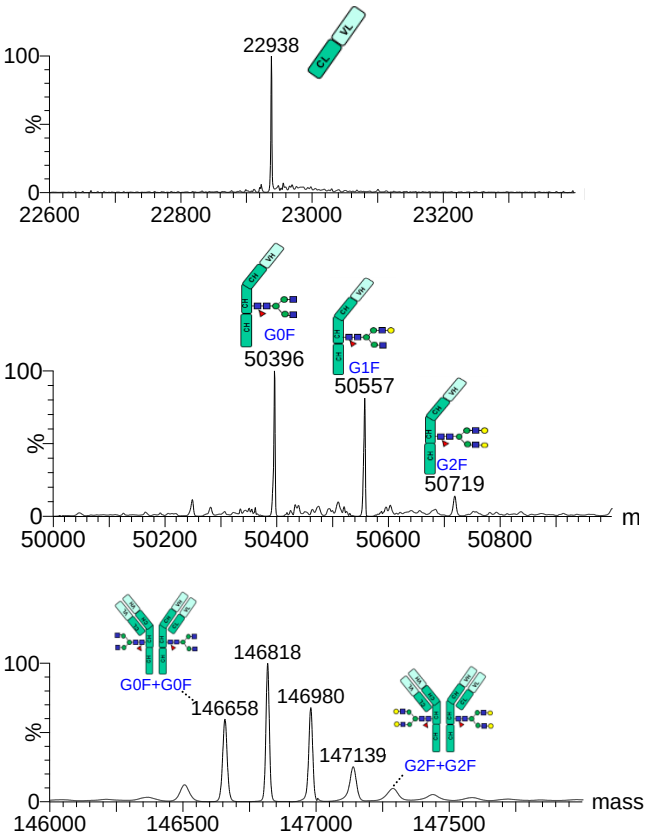
Storage/Stability
Store the lyophilized product at –20 °C.

Reference
1. Beck, A., *et al.*, Biosimilar, biobetter and next generation antibody characterization by mass spectrometry. Anal. Chem., **84(11)**, 4637-4646 (2012).

JK,PRJ,KR,GCY,GA,MAM 01/18-1

Appendices

Figure 1.
Mass spectra



(a) Light Chain, calculated mass: 22,938 Da
Partially reduced, with 2 intact intrachain disulfide bonds (–4 Da)

(b) Heavy Chain, calculated mass: 50,395 Da
Partially reduced, with 4 intact intrachain disulfide bonds (–8 Da)

(c) Intact SigmaMAb, calculated mass: 146,658 Da

Deconvoluted mass spectra of (a) SigmaMAb light chain, (b) SigmaMAb heavy chain, and (c) intact SigmaMAb. The reduction was performed in non-denaturing conditions, where the inter-chain disulfide bonds (which are more susceptible to reduction) will break and produce the light chain and heavy chains, while the intra-chain disulfide bonds within each individual domain remain intact.

Table 1.
The calculated molecular mass of light chains, heavy chains, and intact SigmaMAb with the most abundant glycoforms

Description	Composition	Modification	Average Mass (Da)*	Disulfide bond
Light chain, reduced	$C_{1006}H_{1555}N_{267}O_{333}S_7$	Pyroglutamic acid (Q)	22,942.2	2 intrachain
Heavy chain, reduced	$C_{2181}H_{3393}N_{587}O_{663}S_{16}$ $C_{2237}H_{3485}N_{591}O_{702}S_{16}$ $C_{2243}H_{3495}N_{591}O_{707}S_{16}$ $C_{2249}H_{3505}N_{591}O_{712}S_{16}$	– G0F G1F G2F	48,957.8 50,403.2 50,565.3 50,727.5	4 intrachain
Native, intact product, non-reduced	$C_{6374}H_{9864}N_{1708}O_{1992}S_{46}$ $C_{6486}H_{10048}N_{1716}O_{2070}S_{46}$ $C_{6492}H_{10058}N_{1716}O_{2075}S_{46}$ $C_{6498}H_{10068}N_{1716}O_{2080}S_{46}$ $C_{6504}H_{10078}N_{1716}O_{2085}S_{46}$ $C_{6510}H_{10088}N_{1716}O_{2090}S_{46}$	2 × Pyroglutamic acid (Q) G0F+G0F G0F+G1F G1F+G1F G1F+G2F G2F+G2F	143,767.7 146,658.4 146,820.6 146,982.7 147,144.8 147,307.0	16 (12 intrachain and 4 interchain)

G0F: $GlcNAc_2Man_3GlcNAc_2Fuc$
G1F: $GalGlcNAc_2Man_3GlcNAc_2Fuc$
G2F: $Gal_2GlcNAc_2Man_3GlcNAc_2Fuc$

* Masses based on NIST Physical Reference Data

Figure 2.
Disulfide bonds of SILu™Lite SigmaMab