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Product Information

SILu™Lite SigmaMab K4
Universal Monoclonal Antibody Standard, Human
recombinant, expressed in CHO cells

Catalog Number **MSQC14**
Storage Temperature –20 °C

Product Description

SigmaMab K4 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 K4 is an IgG4 antibody with a Kappa 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).

Human IgG4 antibodies have the capacity to exchange half-molecules, which is commonly called ‘Fab-arm exchange’. SigmaMab K4 has a single amino acid substitution, serine to proline in the hinge region, which reduces Fab arm exchange and stabilizes the molecule.[1]

The antibody sequence has been evaluated by non-reduced and reduced intact mass and peptide mapping.

Each vial of SigmaMab K4 contains 500 μg of antibody lyophilized from a solution of phosphate buffered saline. Vial content was determined by measuring A_{280} and using an extinction coefficient ($E^{0.1\%}$) of 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.

Sequence Information
SigmaMab K4 Heavy Chain:
EVQLVESGGGLVQPGGSLRLSCVASGFTLNNYDMH
WVRQGIGKGLEWVSKIGTAGDRYYAGSVKGRFTISR
ENAKDSLYLQMNSLRVGDAAVYYCARGAGRWAPLG
AFDIWGQGTMVTVSSASTKGPSVFPLAPCSRSTSES
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV
DKRVESKYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLG

SigmaMab K4 Light Chain:
QSALTQPRSVSGSPGQSVTISCTGTSSDIGGYNFVS
WYQQHPGKAPKLMIYDATKRPSGVPDRFSGSKSGN
TASLTISGLQAEDEADYYCCSYAGDYTPGVVFGGGT
KLTVLTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

Preparation Instructions

SigmaMAb K4 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

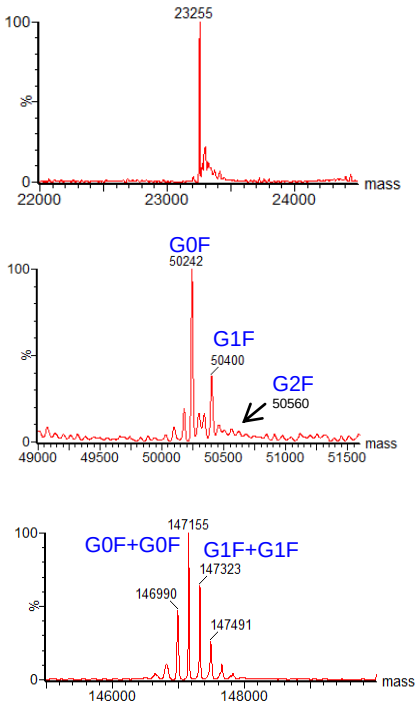
Store the lyophilized product at –20 °C.

Reference

Angal, S., *et al*., Mol. Immunol., **30**(1), 105-108 (1993).

Appendices

Figure 1.
Mass spectra



(a) Light Chain, calculated mass: 23,254 Da
Partially reduced, with 2 intact intrachain disulfide bonds (–4 Da)

(b) Heavy Chain, calculated mass: 50,240 Da
Partially reduced, with 4 intact intrachain disulfide bonds (–8 Da)

(c) Intact SigmaMAb K4, calculated mass: 146,979 Da

Deconvoluted mass spectra of (a) SigmaMAb light chain, (b) SigmaMAb heavy chain and (c) Intact SigmaMAb K4. The reduction was performed in non-denaturing conditions, where the interchain disulfide bonds (which are more susceptible to reduction) will break and produce the light chain and heavy chains, while the intrachain disulfide bonds within each individual domain remain intact.

Table 1.
The calculated molecular mass of light chains, heavy chains of SigmaMab K4 with the most abundant Glycoforms

Description	Composition	Modification	Avg. Mass (Da)*	Disulfide Bond
Light chain, Reduced	$C_{1017}H_{1572}N_{272}O_{339}S_7$	Pyroglutamic acid (Q)	23257.5	2 intrachain
Heavy chain, Reduced	$C_{2171}H_{3364}N_{582}O_{665}S_{17}$	–	48802.5	4 intrachain
	$C_{2227}H_{3456}N_{586}O_{704}S_{17}$	G0F	50247.9	
	$C_{2233}H_{3466}N_{586}O_{709}S_{17}$	G1F	50410.0	
	$C_{2239}H_{3476}N_{586}O_{714}S_{17}$	G2F	50572.2	
Native. Intact product, non-reduced	$C_{6376}H_{9840}N_{1708}O_{2008}S_{48}$	2 × Pyroglutamic acid (Q)	144200.1	16 (12 intrachain and 4 interchain)
	$C_{6488}H_{10024}N_{1716}O_{2086}S_{48}$	G0F+G0F	146978.7	
	$C_{6494}H_{10034}N_{1716}O_{2091}S_{48}$	G0F+G1F	147140.9	
	$C_{6500}H_{10044}N_{1716}O_{2096}S_{48}$	G1F+G1F	147303.0	
	$C_{6506}H_{10054}N_{1716}O_{2101}S_{48}$	G1F+G2F	147465.2	
	$C_{6512}H_{10064}N_{1716}O_{2106}S_{48}$	G2F+G2F	147627.3	

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 K4