

Light chain isotype and antibody-specificity impact on virus neutralization

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Supplementary data

Table S1: Primer list to generate heavy and light chain constant domains of antibody isotypes with flanking BsmBI (CGTCTC), facilitating introduction of variable fragment with BsaI (GGTCTC) restriction sites

Primers	Sequence
IgG1(-Fv) -F1	GAGCGTCTCTAGGTA <u>GAGACC</u> GGGATCCGGTCTCTGCATCAACCAAAGGTCC
IgG1-R1	GCACGTCTCAAAGCTCACTTTCCAGGAGAAAGAG
κLc(-Fv) -F1	GAGCGTCTCTAGGTA <u>GAGACC</u> GGATCCGGTCTCTCGTACTGTTGCAGCTC
κLc-R1	GCACGTCTCAAAGCTTAGCATTCACCTCGATTAAAG
λLc(-Fv) -F1	GAGCGTCTCTAGGTA <u>GAGACC</u> GGGATCCGGTCTCTCAACCTAAGGCTGCTCC
λLc-R1	TATACGTCTCTAAGCCTAGCTGCACTCAG

Table S2: Yield of purified mAbs

mAb	Yield (μg/g)
P5C3IgG1 κ	220
P5C3IgG1 λ	300
H4IgG1 κ	250
H4IgG1 λ	220

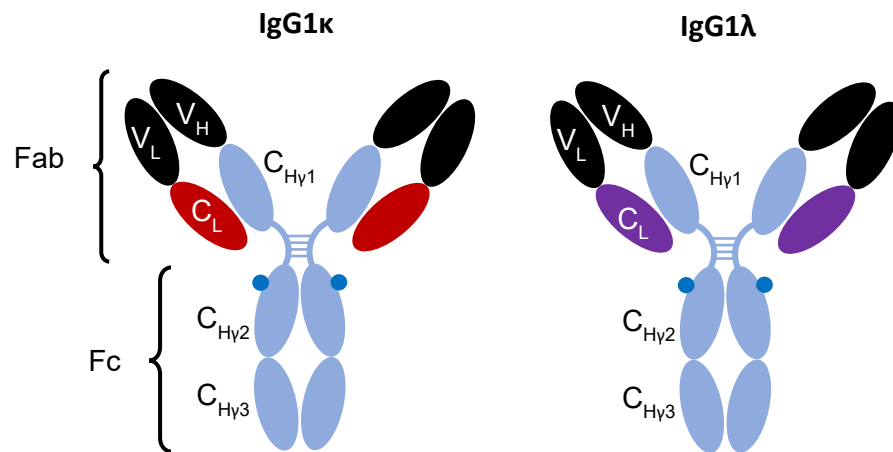


Figure S1: Schematic representation of IgG1κ and IgG1λ. VL: variable light; VH: Variable heavy; CL: constant light; CH_{γ1}-CH_{γ3}: constant heavy gamma chain domain1-3; Fab: Antigen binding fragment; Fc: Crystallizable fragment. Blue dot: Conserved N-glycosite

H4-IgG1Hc

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWRQAPGQGLEWMGRINPNSGGTNYA
QKFQGRVTMTDRDTSISTAYMELSRLRSDDTAVYYCARVPYCSSTSCHRDWYFDLWGRGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**NSTY**RVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

H4-IgGκLc (allotype Km3)

DIQMTQSPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKPGQSPQLLIYTLSYRASGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRIEFPLTFGGGKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

H4-IgGλLc

DIQMTQSPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKPGQSPQLLIYTLSYRASGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRIEFPLTFGGGKVEIKQPKAAPSVTLFPPSS
EELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQW
KSHRSYSCQVTHEGSTVEKTVAPTECS

P5C3-IgG1Hc

QMQLVQSGPEVKKPGTSSVKVSCKASGFTFTSSAVQWVRQARGQRLEWIGWIVVGSGNTDYA
QQFQERVITRDMSTSTAYMELSSLGSEDTAVYYCAAP**NC**SGGSCYDGFDLWGQGTMTVTS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**NSTY**RVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

P5C3-IgGκLc (allotype Km3)

EIVLTQSPGTLSPGERATLSCRGSQSVRSSYLGWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

P5C3-IgGλLc

EIVLTQSPGTLSPGERATLSCRGSQSVRSSYLGWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRS
YSCQVTHEGSTVEKTVAPTECS

Figure S2: Peptide sequences of IgG1κ and IgG1λ Black: variable heavy and light chain fragments, Blue: constant heavy chain, Red: constant kappa light chain, Purple: constant Lambda light chain, N-glycosite highlighted in bold and Red.

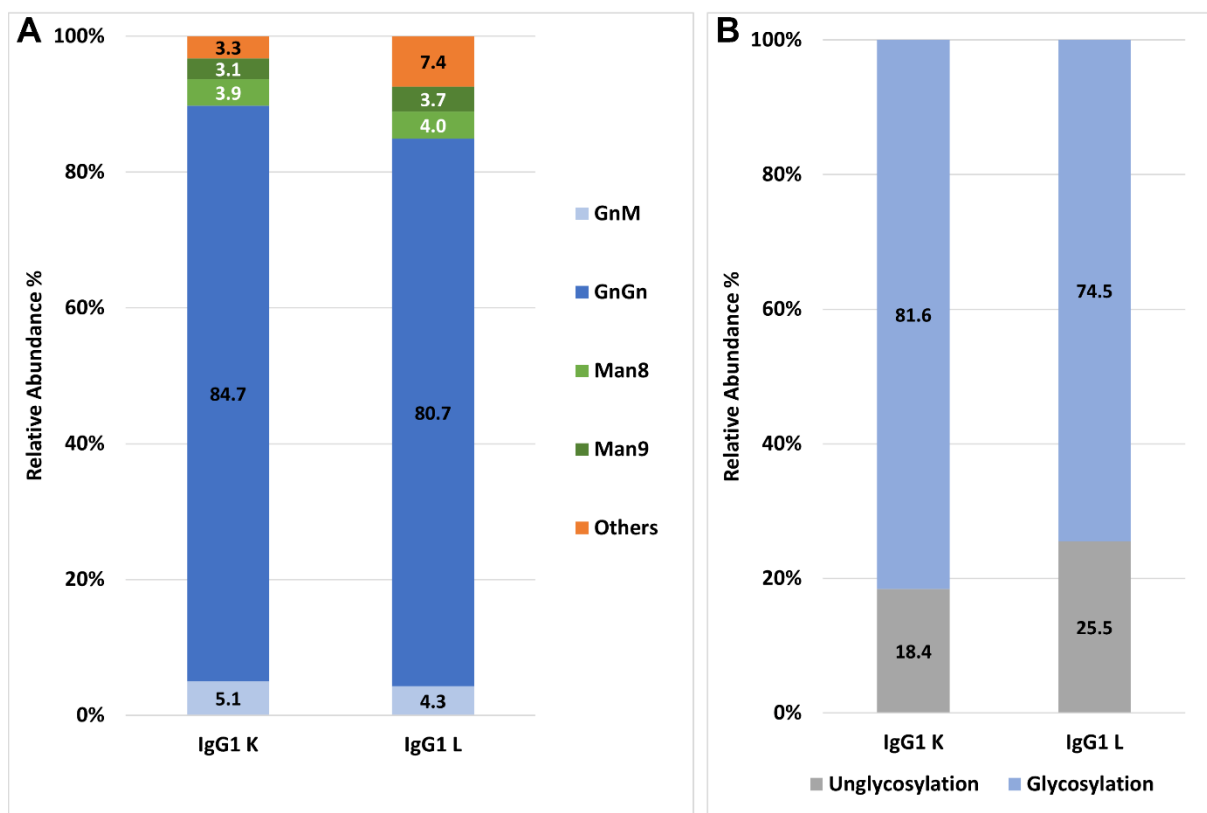


Figure S3: A. LC-ESI-MS/MS-derived N-glycosylation profiles of purified P5C3-IgG1 κ and λ . Bars represent the relative abundance (%) of glycoforms present at the Fc GS. Blue: complex GlcNAc-terminating N-glycans (GnM-GnGn); green: mannosidic N-glycans (Man8-Man9). Orange: combines detected glycans below 3%. **B.** Occupied (blue) and non-occupied glycosite (grey) in %. Nomenclature according to Altmann et al 2024.