Attribute	Characterization Methods
Biochemical Characterization	
Primary Structure	• Peptide map/MS/MS using different enzymes
Oxidized variants and deamidated species	• Sites and amounts of oxidized and deamidated species by peptide map/MS/MS • Total deamidation (isoAsp) by IsoQuant Assay • Oxidized Mabs by hydrophobic interaction HPLC
Aggregates/Association States	• Quantitation and assessment of reversibility by SEC • Characterization by SEC-MALLS detection* • Analytical Ultracentrifugation* • Dynamic Light Scattering* • SDS-PAGE reduced/non-reduced (Coomassie Stained)
Fragments	• Size exclusion HPLC • SDS-PAGE reduced/non-reduced • Intact and reduced mass to verify MW and deduce structure • N-terminal sequencing of bands cut from gel to deduce identity*
Glycovariants	• Intact mass distribution of glycovariants • Glycan maps of PNGaseF-released N-glycans • Sialic acid content (NANA and NGNA) • N-linked and O-linked glycans by peptide map/MS • Site-specific glycan analysis
Disulfide linkage analysis	• Peptide map/MS ± reducing and carboxymethylating agents • Free thiol content by DTNB colorimetric assay. • MS analysis of Fabricator cleaved Fc and Fab segments of Mabs and fusion proteins.
Charge variants (chemical or physical)	• Ion exchange HPLC • cIEF*
Biophysical Characterization	
Secondary structure	• Far UV CD spectroscopy with data fitting* • FTIR*
Tertiary structure	• Near UV CD spectroscopy* • Fluorescence spectroscopy*
Melting temperature (structural stability)	• T_m and ΔH by differential scanning calorimtery
Impurities	
Reidual Host cell proteins	• ELISA • 2D SDS-PAGE and Mass Spec analysis of coverage • Mass fingerprint of HCPs
Residual Host cell DNA	• qPCR
Column resin leachate	• e.g. Protein A (ELISA), residual heparin, etc.
Process additives	• RP-HPLC, quantitative MS (depending on the type of additive)

Product-related Variants/Impurities	• SDS-PAGE (Silver stained)
Biological Activity	
Ligand binding (as appropriate)	• ELISA • Biacore • Label-free binding kinetics by ForteBio
Potency	• Product-specific cell based bioassays • ADCC & CDC (for antibodies)
Fcγ binding (for antibodies)	• CHO-expressed Fc receptor cell based binding assay (FACS)
FcRn binding (for antibodies)	• CHO-expressed FcRn cell based binding assay (FACS)
Safety	
Immunogenicity	• Cytokine release from PBMCs or in whole blood or specific cells
Particulates	• Subvisible particles by light obscuration and microflow imaging* • Visual appearance test for color, clarity, and particles.