Checklist for Preclinical Studies

CATALYZE
Accelerating Translation

Biologics

Notes

This workbook outlines technical/CMC, ADME, primary pharmacology, secondary pharmacology, and bioanalytical studies typically completed before a Pre-IND for biologics.
Note: Completion of all studies is not required; please indicate which studies have been completed.

Key references: ICH Q6B (specifications & characterization), ICH S6(R1) (preclinical safety for biotech-derived pharmaceuticals), ICH S7A/S7B (pharmacology & safety pharmacology), USP <71>/<85> (sterility & endotoxin), FDA Bioanalytical Method Validation Guidance, and FDA Immunogenicity Assessment Guidance.

Phase-appropriate expectations: fit-for-purpose characterization and assays at Pre-IND, with GLP-compliant pivotal studies for IND submission as applicable; species selection must be pharmacologically relevant to the target.

Category	Item/Study	Purpose	Key Outputs / Acceptance Criteria	Species	Model	Matrix	Design Considerations	Reference / Guideline	Notes
Technical/ CMC	☐Molecular Identity & Characterization	Confirm biologic identity and structure (primary sequence, PTMs, disulfides)	Intact mass within tolerance; >95% sequence coverage; expected PTMs					ICH Q6B	Use orthogonal methods (LC–MS, peptide mapping)
Technical/ CMC	☐Purity & Variants (Charge & Size)	Profile charge and size heterogeneity	Main peak purity; aggregates below phase-appropriate limits					ICH Q6B	IEX/cIEF; SEC/SEC–MALS
Technical/ CMC	☐Glycosylation	Characterize glycan species impacting function/stability	Relative abundance of key glycoforms					ICH Q6B	Released N-glycan UHPLC/LC–MS
Technical/ CMC	☐Functional Characterization	Verify binding and cell-based biological activity	KD/kinetics; EC50/IC50 within expected ranges					ICH Q6B	SPR/BLI/ELISA and potency assay (fit-for-purpose)
Technical/ CMC	☐Impurities & Safety (Compendial)	Quantify process-related impurities and microbial safety	Sterile (USP <71>); endotoxin within limits (USP <85>); HCP/DNA below action limits					USP <71>/<85>; FDA CBER	Product-specific suitability for LAL
ADME	☐Biodistribution	Determine tissue distribution and target organ exposure	Tissue concentration profiles; exposure vs plasma	Rodent ± NHP (MoA-dependent)			Radiolabeled/ tagged biologic; imaging/ quantitative assays	ICH S6(R1)	Critical for restricted/BBB targets
ADME	☐Excretion/ Clearance Pathways	Identify dominant clearance routes	% recovery by route; catabolic fate	Rodent			Urine/feces collections (if feasible)	ICH S6(R1)	Large biologics often via proteolysis
ADME	☐Metabolism/ Degradation	Assess catabolic fragments and potential activity	Fragment profiles; impact on PK/PD	Rodent/NHP			Sensitive LC–MS/ELISA; time-course	ICH S6(R1)	Link immunogenicity risk
Primary Pharmacology	☐In vitro Target Engagement	Demonstrate specific binding/activation/ inhibition at intended target	Potency (EC50/IC50), Emax		Biochemical/ cellular assays		Concentration-response; controls	ICH S7A (pharmacology)	Define translational biomarkers
Primary Pharmacology	☐In vivo Pharmacology (PoC Efficacy)	Confirm pharmacological activity in relevant model	Statistically significant efficacy; biomarker changes		Disease-relevant animal model		Dose-response; endpoints; PK/PD linkage	ICH S6(R1); FDA nonclinical guidance	Species must be pharmacologically relevant

Secondary Pharmacology	☐Off-target Screening	Identify unintended pharmacological effects	No concerning off-target signals		Panels (receptors/enzymes) where relevant		Tiered approach based on MoA	ICH S7A	Scope tailored to biologic modality
Secondary Pharmacology	☐Safety Pharmacology (Core Systems)	Evaluate acute effects on vital organ systems	No unsafe acute effects at relevant exposures		CV/respiratory/ CNS assessments (as applicable)		Align with tox; telemetry/respiratory monitoring	ICH S7A; S7B (if applicable)	Extent depends on MoA/clinical risk
Bioanalytical	☐PK Method (Ligand-binding or LC–MS/MS)	Quantify drug in biological matrices	Validated fit-for-purpose PK assay			Serum/ plasma	Selectivity, sensitivity, accuracy ±15% (±20% at LLOQ)	FDA Bioanalytical Method Validation	Assess matrix effects, dilution integrity
Bioanalytical	☐ADA/ Immunogenicity Assay	Detect anti-drug antibodies and assess impact on PK/PD	ADA incidence/titers; neutralizing vs binding			Serum/ plasma	Tiered screening → confirmatory → titer	FDA Immunogenicity Assessment Guidance	Coordinate sampling with PK
Bioanalytical	☐Biomarker Assays (PD)	Measure pharmacodynamic biomarkers supporting MoA	Robust PD readouts			Blood/tissue	Clinical translatability; qualified assays	ICH S7A; FDA biomarker guidance	Supports dose selection
Technical/ CMC	☐Potency Assay	Confirm potency of DS to have consistent dose even from different batches	Numerical value relative to reference standard or control				Qualified assay, eventual validation	ICH Q6B	When an appropriate potency assay is used for the drug product (section IV.B.4), an alternative method (physicochemical and/or biological) may suffice for quantitative assessment at the drug substance stage.
Technical/ CMC	☐Quantitative Assay	Confirm DS content and amt of DS in the DP	Numerical value, will become a release specification				Qualified assay, eventual validation	ICH Q6B	In cases where product manufacture is based upon potency, there may be no need for an alternate determination of quantity.