Solutions supporting quality assessment for monoclonal antibody (mAb) development and manufacturing

USP offers a system of well-characterized quality solutions to aid in mAb development and manufacturing

- Provide a means to evaluate and monitor performance of assays which measure critical quality attributes (CQAs) of biotherapeutics
- Facilitate adoption of leading edge analytical technologies by providing well characterized standards
- Assist in reducing assay variability by providing materials for assay controls

USP mAbs are non-compendial reference standards (RSs) that are supported by USP quality and lifecycle management including ongoing suitability for use studies for the lifetime of the RSs.

All USP mAb RSs are recombinant humanized IgG1s expressed in Chinese hamster ovary (CHO) cell culture (currently the most common cell line used for mAb manufacturing) and are manufactured using industry standard upstream production and downstream purification.

The USP mAb RSs have been rigorously tested and evaluated during multi-laboratory studies using the methods described in USP General Chapters *<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*, *<210> Monosaccharide Analysis*, and *<212> Oligosaccharide Analysis*. Assays performed on the USP mAb RSs include: SE-HPLC, IgG Content analysis by CE-SDS, Intact Protein Mass, N-linked Oligosaccharide analysis by CE-LIF and HILIC LC-FLR-MS, and N-glycan analysis by CE-LIF. Post-translational modification analysis (such as glycosylation, N-terminal pyroglutamate and C-terminal lysine deletion) were also studied as part of the RSs evaluation.

The extensive characterization of the three mAb RSs with different physicochemical properties provides you the option to select the mAb that best reflects the key attributes you want to assess for your specific analytical needs/purposes, saving time and costs associated with in-house evaluation of the RSs.

NEW! Host Cell Proteins (HCPs)

Demonstrating clearance of HCPs is required for mAbs. USP now supports HCP analysis by mass spectrometry with our new Recombinant CHO PLBL2 protein. This well characterized material can be used as a standard or control in a PLBL2 ELISA or mass spectrometry-based workflows to monitor the clearance of this HCP from your therapeutic product. HCP analysis by MS is supported by <1132.1> *Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry**, in PF 49(3).

Characterization Data

- Capillary isoelectric focusing (cIEF)
- Imaged capillary isoelectric focusing (icIEF)
- SE-HPLC (Size exclusion – High-performance liquid chromatography)
- Capillary electrophoresis sodium dodecyl sulfate (CE-SDS) (reduced and non-reduced)
- Intact (Protein) Mass by Spectrometry
- N-Glycan analysis using Capillary electrophoresis – Laser Induced Fluorescence detection (CE-LIF) and Hydrophilic interaction chromatography – Fluorescence – Mass spectrometry (HILIC-FLR-MS)

Example Applications

- Internal assay control
- Independent control material for method development
- Standardization of physico chemical testing
- Training activities
- Method transfer activities
- Optimization of platform methods (e.g., glycan analysis)
- Higher order structure

USP's MAM knowledge hub

USP is evaluating the use of RSs in additional applications, including Multi-Attribute Method (MAM). To learn more about **USP's MAM Knowledge Hub**, an online community of scientists and experts dedicated to accelerating MAM scientific knowledge, register at mam.usp.org.

For more information:

Visit https://www.usp.org/biologics/mabs

usp.org/biologics

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Table 1. General information for the three non-compendial USP mAb Reference Standards

	USP mAb 001, monoclonal IgG1	USP mAb 002, monoclonal IgG1	USP mAb 003, monoclonal IgG1
CAS #	174722-31-7	216974-75-3	912628-39-8
MW	~147,000 Da	~150,000 Da	~146,000 Da
Theoretical pI*	8.7	8.1	8.1
Experimental pI (cIEF)**	9.2	7.8	7.7
Experimental pI (icIEF)**	9.2	7.9	7.8
Package size	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)

** Calculated using ProtParam (ExPASy) without glycosylation*
***Per USP In-house methods, see charge variant application note for more details.*

Supporting Resources

USP Education Courses	• What's new in Biologics? Focus on USP's Monoclonal Antibody Reference Standards • USP-NF General Chapter <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies* • Development and Characterization of Monoclonal Antibody Therapeutics • Characterization of Biotherapeutics • Assays to Evaluate Fragment Crystallizable (Fc)-Mediated Effector Function • Analysis of Charge Variants in Biologics
USP Chapters	<129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies* <210> *Monosaccharide Analysis* <212> *Oligosaccharide Analysis* <507> *Protein Determination Procedures* <1055> *Biotechnology-Derived Articles-Peptide Mapping: Identity* <736>, <1736> *Mass Spectrometry Chapters* <1132> *Residual Host Cell Protein Measurement in Biopharmaceuticals* <509> *Residual DNA Testing* <791> *pH* <631> *Color and Achromicity* <71> *Sterility Tests* <85> *Bacterial Endotoxin Tests* <787>, <790> *Subvisible and Visible Particulate Matter*

References

1. USP Reference Standard Monoclonal Antibodies: Tools to Verify Glycan Structure
2. Higher-Order Structure (HOS) Characterization of USP Monoclonal Antibody Reference Standards
3. Development of Standards for Cation Exchange Chromatography Column Qualification
4. Charge Variant Analysis of USP Monoclonal Antibody Reference Standards
5. The role of public standards in the development of biosimilars

Excipients

USP offers monographs and the following reference standards decribed therein, for excipients used in the mAb manufacturing process or present in mAb formulations. Pharmaceutical excipients are substances other than the active pharmaceutical ingredient (API) that have been appropriately evaluated for safety and are intentionally included in a drug delivery system. Excipients are important to study in a drug as these may have some biological activity even though they are not intended to exert therapeutic effects.

Citric acid	L-Histidine	Mannitol	Sodium chloride
Dextrose	L-Histidine monohydro-chloride monohydrate	Monosodium glutamate	Sodium citrate
Glacial acetic acid	L-Lysine hydrochloride	Pentetic acid	Sorbitol
Glutamic Acid	L-Methionine	Polysorbate 20	Sucrose
Glycine	L-Phenylalanine	Polysorbate 80	Trehalose
Insulin	L-Threonine	Sodium acetate trihydrate	

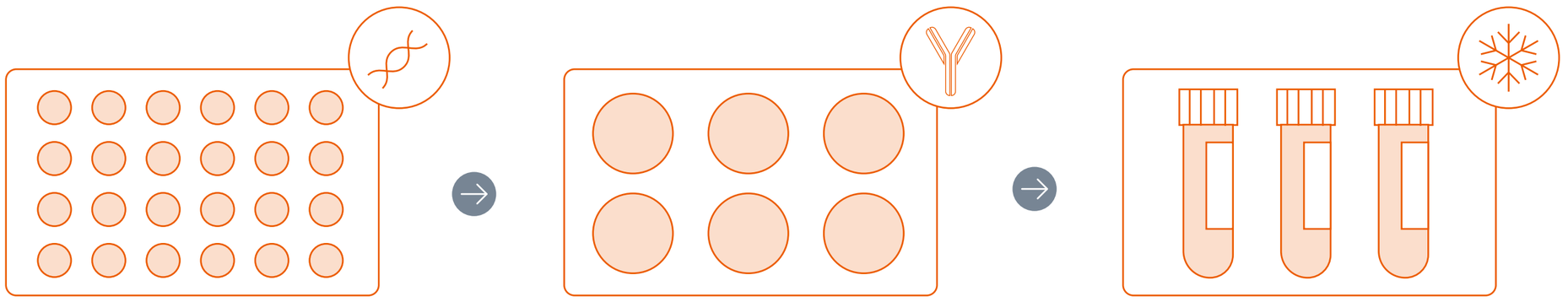
Please visit our site for a full list of all available excipients

About USP

USP is an independent scientific organization that collaborates with the world's top experts in health and science to develop quality resources and standards for medicines, dietary supplements, and food ingredients. Through our resources, standards, advocacy and education, USP helps increase the availability of quality medicines, supplements and foods for billions of people worldwide.

Ensuring quality in monoclonal antibody therapeutics with USP standards

1 CELL LINE DEVELOPMENT & EXPANSION



Cell Line Selection ⓘ
<1048>

Clone & Banking ⓘ
<1042>* <1050> <1237>

Expansion & Cryopreservation ⓘ
<1044>

ANTIBODY CHARACTERIZATION

Identity & Structure ⓘ 2
<736> *Mass Spectrometry*
<1736> *Intact & Reduced Mass*
<1055> *Peptide Mapping*
<210> <212> <1084> *Glycan Profiling* 1
<1052> *Amino Acid Analysis*
<1054> *IEF (pI determination)*
<1102> <1103> *Identity ELISA*
<1105> *Surface Plasmon Resonance*
<891> *Thermal Analysis*
<761> *NMR Spectroscopy*
<1853> *Fluorescence Spectroscopy*
<1430.3> *Dynamic Light Scattering*

General Properties ⓘ
<507> <1057> *Protein Concentration*
<785> *Osmolality*
<791> *pH*
<631> *Color/Clarity*
<790> *Appearance*

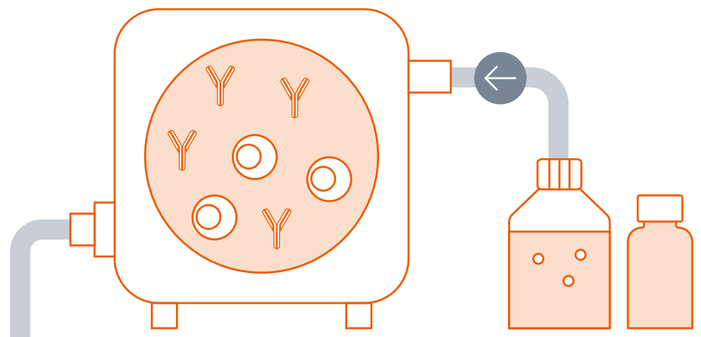
Product-Related Impurities ⓘ
<129> *SEC HPLC, CEX/cIEF, CE SDS, Aggregation & Degradation* 2
<621> *Chromatography*
<1053> *Capillary Electrophoresis*

Process-Related Impurities ⓘ
<1132> *Host Cell Proteins (HCP)*
<1132.1> *HCP by Mass Spec (in PF 49(3))*
<509> *Host Cell Residual DNA (HCD)* 3
<1130> *Residual DNA Testing*

Potency ⓘ
<111> *Design & Analysis of Biological Products*
<1032> <1033> <1034> *Potency Bioassay*
<1108> *FcRn*

Contaminants ⓘ
<85> *Endotoxins* 4
<63> *Mycoplasma*
<1237> *Virus Testing*
<61> <62> <1229.3> *Bioburden*

2 UPSTREAM PRODUCTION



Bioreactors ⓘ
<665>* <1665>*

Raw Material & Cell Substrate Qualification ⓘ
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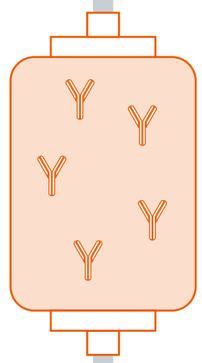
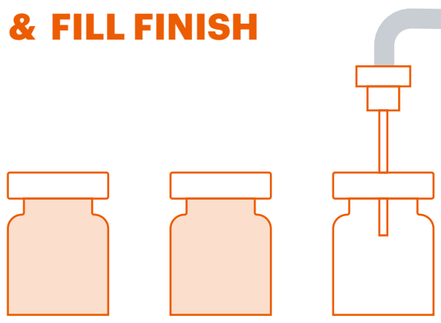


3 DOWNSTREAM PRODUCTION

Purification
Protein A
IEX Chromatography

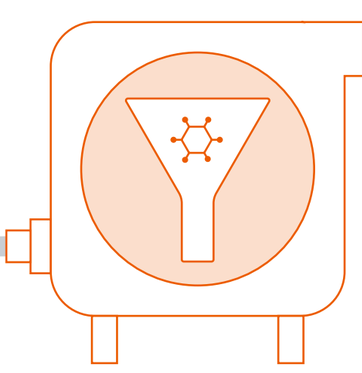
4 FORMULATION & FILL FINISH



Formulation ⓘ
<1059> <1231>

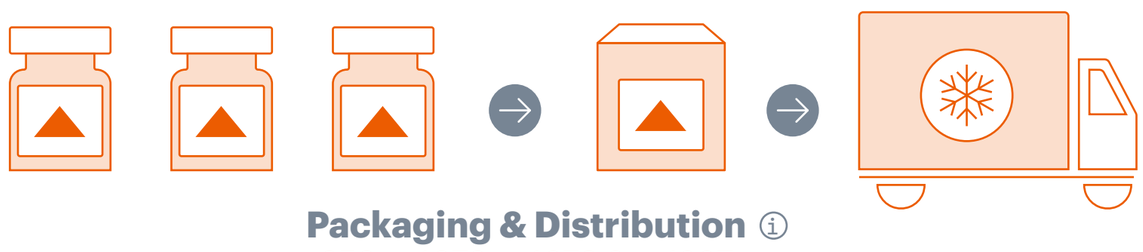
Sterile Filtration & Filling ⓘ
<1> <71> <787> <788> <1116> <1211> <1229.4> <1787>

Stability ⓘ
<1049> <1049.1> *Proposed*



Purification ⓘ
Virus Inactivation
Filtration
<1050> <1050.1>

5 PACKAGING & DISTRIBUTION



Packaging & Distribution ⓘ
<659> <1079> <1079.2> <1207>

Physical Materials

1 Glycan Profiling
N-Acetylneuraminic Acid (Cat # 1612619)
N-Glycolylneuraminic Acid (Cat # 1294284)
Oligosaccharide System Suitability Mixture A (Cat # 1478210)
Oligosaccharide System Suitability Mixture B (Cat # 1478221)
Oligosaccharide System Suitability Mixture C (Cat # 1478232)
Oligosaccharide System Suitability Mixture D (Cat # 1478243)

2 Monoclonal Antibodies
Monoclonal IgG Suitability (Cat # 1445550)
Monoclonal IgG1, mAb 001 RS (Cat # 1445539)
Monoclonal IgG1, mAb 002 RS (Cat # 1445547)
Monoclonal IgG1, mAb 003 RS (Cat # 1445595)

3 Impurities
PLBL2 HCP (Cat # 1582716)
CHO Genomic DNA (Cat # 1130710)
E. coli Genomic DNA (Cat # 1231557)

4 Adventitious Agents
Endotoxin (Cat # 1235503)

Download the Complimentary Chapter <129>
go.usp.org/l/323321/2018-10-16/xxzsl

For more details contact:
USPBiologics@USP.org

* In-Process Revision

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