

Biologics Characterisation & Specification

Write characterisation, impurities, specification and justification, and reference standards for a protein or antibody, with impurity levels per dose and the endotoxin limit calculated from the dose.

WRITES

3.2.S.3.1
3.2.S.3.2
3.2.S.4.1
3.2.S.4.5
3.2.P.5.1
3.2.P.5.6
3.2.S.5

PRODUCT TYPE

Biologics
(biotechnology
products)

GUIDANCE IT FOLLOWS

ICH Q6B, ICH Q5C,
ICH Q2(R2), ICH
M4Q(R1) 3.2.S.3
and 3.2.S.4; FDA
Points to Consider
in the Manufacture
and Testing of
Monoclonal
Antibody Products
(1997); USP <85>,
USP <71>, USP
<787>/<788>, USP
<790>, USP <697>

TIME TO LEARN

About 20 minutes
with the example

Open the tool at <https://app.cmcfy.com/app/biologics-characterisation.html>. This guide is also online at <https://cmcfy.com/guides/biologics-characterisation.html>

1. What this module does

The module writes 3.2.S.3.1, 3.2.S.3.2, the specification and its justification (3.2.S.4.1 and 4.5, or 3.2.P.5.1 and 5.6) and 3.2.S.5.

It checks your characterisation against the ICH Q6B list (primary structure, post-translational modifications, glycans, higher-order structure, variants, biological activity), converts host cell protein and DNA to amounts per dose, and calculates the endotoxin limit from the dose and route (K/M).

What you get

- Word or PDF files for characterisation, impurities, specification, justification and reference standards.

- A summary file for the QOS Composer & Dossier Assembler.

2. Before you start

Have these ready. You can still start with the example data and replace it piece by piece.

- Maximum dose, route, shortest infusion time and protein concentration.
- Characterisation results by attribute and method, with the lots tested.
- Host cell protein (ng/mg), residual DNA (pg/mg) and leached Protein A (ng/mg), with the HCP assay and its coverage; other process-related and product-related impurities.
- Tests, methods and acceptance criteria, and the lots (toxicology, clinical, PPQ) that support them.
- Primary and working reference standards and the protocol for new ones.

Your data stays on your computer. The bar under the title counts every network request the page makes; it stays at 0 because your data never leaves your computer. [Download offline](#) [copy](#) saves a single file that opens from your own computer with all network access blocked, for air-gapped PCs.

3. Step by step with the example

Open the tool at app.cmcfy.com/app/biologics-characterisation.html. The example is loaded when the page opens, so every panel already has realistic data. Follow the steps to see how it works, then replace the example with your own data.

1 Enter product and dose

Choose drug substance or drug product specification, the route, the maximum dose (1500 mg in the example), the shortest infusion time and the concentration. These drive the per-dose calculations.

CMCfy | **Biologics Characterisation & Specification** | Projects | All modules | Training | Security

Runs entirely on this device | **Training guide** | Protocol builder

PROJECTS Keep this work in a project, with version history and your company logo on every report. Choose projects folder Keep in this browser

- Network requests since load: 0**
Counted live by your browser. Your data never leaves this device.
- Works offline**
Everything the tool needs is inside this page.
- Locked offline copy**
Save a single file that opens from your own computer with all network access blocked.

3.2.S.3.1 3.2.S.3.2 3.2.S.4.1 3.2.S.4.5 3.2.P.5.1 3.2.P.5.6 3.2.S.5

Save project Open project Start blank Load example

Write characterisation, impurities, specification and justification, and reference standard sections for a biotechnology product. The tool checks the ICH Q6B attributes, calculates impurity levels per dose and the endotoxin limit from the dose, and compares them with your proposed criteria.

Guidance: ICH Q6B, ICH Q5C, ICH Q2(R2), ICH M4Q(R1) 3.2.S.3 and 3.2.S.4; FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products (1997); USP <85>, USP <71>, USP <787>/<788>, USP <790>, USP <697>

1 · PRODUCT AND DOSE

Product
CMB-201 (humanised IgG1 monoclonal antibody)

Submission region
US: FDA (NDA, ANDA, BL)

Specification for
Drug product (3.2.P.5)

Route
Intravenous

Maximum dose (mg)
For dosing per kg, give the dose for a 70 kg adult.
1500

Shortest infusion time (h)
Bolus or 1 h or less: enter 1.

Protein concentration (mg/mL)
100

RESULT

1 expected attribute or test missing

Endotoxin limit from the dose: 0.23 EU/mg (K = 5 EU/kg, M = 21.43 mg/kg/h). Residual DNA: 0.75 ng per dose.

SPECIFICATION	HCP PER DOSE	DNA PER DOSE
Drug product	6.75 µg	0.75 ng
ENDOTOXIN LIMIT (K/M)	0.23 EU/mg	

ICH Q6B CHARACTERISATION

ATTRIBUTE	METHOD	RESULT
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2 Enter characterisation and impurities

One row per attribute studied. Enter HCP, DNA and Protein A as the highest level across lots.

3 Read the result

The headline reports missing Q6B attributes, and the note gives the endotoxin limit from the dose (0.23 EU/mg with K = 5 EU/kg and M = 21.43 mg/kg/h) and residual DNA per dose (0.75 ng). The facts show HCP per dose (6.75 µg).

RESULT

1 expected attribute or test missing

Endotoxin limit from the dose: 0.23 EU/mg (K = 5 EU/kg, M = 21.43 mg/kg/h). Residual DNA: 0.75 ng per dose.

SPECIFICATION	HCP PER DOSE	DNA PER DOSE
Drug product	6.75 µg	0.75 ng
ENDOTOXIN LIMIT (K/M)	0.23 EU/mg	

4 Check the specification against Q6B and the lots

The tables show which expected tests are in the specification and whether every supporting lot meets the proposed criteria.

ICH Q6B CHARACTERISATION		
ATTRIBUTE	METHOD	RESULT
Amino acid sequence	Peptide mapping (trypsin and Lys-C) with LC-MS/MS	100% sequence coverage; matches the expected sequence
Molecular mass	LC-MS	148,054 Da (G0F/G0F), matches theoretical within 2 Da
N- and C-terminal sequence	Peptide mapping	N-terminal pyroglutamate 98%; C-terminal lysine 4%
Disulfide bonds and free thiols	Non-reduced peptide map	16 disulfide bonds as expected; free thiol < 0.1 mol/mol
Post-translational modifications	Peptide mapping	Deamidation HC N386 2.1%; Met oxidation HC M255 1.8%
Glycosylation	HILIC-FLD of released glycans	G0F 42%; G1F 35%; G2F 8%; afucosylated 6%; high mannose 3%
Higher-order structure	Far- and near-UV CD, FTIR, DSC	Consistent with IgG1 β -sheet fold; Tm1 71 °C, Tm2 83 °C
Size variants	SEC-HPLC; CE-SDS (reduced and non-reduced); SV-AUC	Monomer 99.1%; HMW 0.8%; LMW 0.1%
Charge variants	icIEF; CEX-HPLC	Main 68%; acidic 24%; basic 8%
Target binding	SPR	KD 0.4 nM
Biological activity (cell-based)	Cell-based reporter assay	Relative potency 98%
Effector functions (Fc)	Fc γ R1IIa binding (SPR), ADCC reporter assay, C1q binding, FcRn binding	ADCC active; CDC low

ICH Q6B characterisation coverage.

5 Resolve alerts

In the example a minor alert notes that the glycan profile is not in the drug product specification; add it or justify why it is controlled elsewhere.

ALERTS TO RESOLVE

All blocking alerts are fixed, justified or accepted. Minor alerts are listed in the Word files.

MINOR

No glycan profile (where it affects activity) test in the drug product specification.

Specification · ICH Q6B 6.3

Add glycan profile (where it affects activity), or justify why it is not needed.

Justification

Accept justification

Preview and download

Preview each section, read the review and download.

Preview and review before download

Close

Review
Checked on this device: gaps in the data, gaps in the report against ICH and regional expectations, and differences from the protocol.

1 to fix 1 recommended

DATA AND INPUTS (1)

No glycan profile (where it affects activity) test in the drug product specification.
Add glycan profile (where it affects activity), or justify why it is not needed.
recommended · Specification · ICH Q6B 6.3 **Ignore**

REPORT CONTENT (1)

Placeholder left in the report: [State the conclusion: the structure is consistent with the expected molecule, and which...]
Replace the placeholder with the real information, or delete the sentence if it does not apply.
major **Ignore**

Compare with the protocol
Load or paste the approved protocol. Every requirement and acceptance criterion in it is looked for in the report.
Paste the protocol text here, or load the file.

3.2.S.3.1 Elucidation of structure and other characteristics
CMB-201 (humanised IgG1 monoclonal antibody) · US (FDA)

Attribute	Method	Result	Lots
Amino acid sequence	Peptide mapping (trypsin and Lys-C) with LC-MS/MS	100% sequence coverage; matches the expected sequence	RS-201-01, DS-201-PPQ1
Intact and reduced molecular mass	LC-MS	148,054 Da (GOF/GOF), matches theoretical within 2 Da	RS-201-01
N- and C-terminal sequence	Peptide mapping	N-terminal pyroglutamate 98%; C-terminal lysine 4%	RS-201-01
Disulfide bonds	Non-reduced peptide map	16 disulfide bonds as expected; free thiol < 0.1 mol/mol	RS-201-01
Post-translational modifications	Peptide mapping	Deamidation HC N386 2.1%; Met oxidation HC M255 1.8%	RS-201-01, DS-201-PPQ1
N-glycan profile	HILIC-FLD of released glycans	GOF 42%; G1F 35%; G2F 8%; afucosylated 6%; high mannose 3%	RS-201-01, PPQ1-3
Higher-order structure	Far- and near-UV CD, FTIR, DSC	Consistent with IgG1 β-sheet fold; Tm1 71 °C, Tm2 83 °C	RS-201-01
Size variants	SEC-HPLC; CE-SDS (reduced and non-reduced); SV-AUC	Monomer 99.1%; HMW 0.8%; LMW 0.1%	PPQ1-3
Charge variants	icIEF; CEX-HPLC	Main 68%; acidic 24%; basic 8%	PPQ1-3
Target binding	SPR	KD 0.4 nM	RS-201-01
Biological activity	Cell-based reporter assay	Relative potency 98%	PPQ1-3
Fc effector functions	FcγRIIIa binding (SPR), ADCC reporter assay, C1q binding, FcRn binding	ADCC active; CDC low	RS-201-01

CMB-201 (humanised IgG1 monoclonal antibody) was characterised by state-of-the-art methods covering the ICH Q6B attributes listed below, using the reference standard and drug substance lots from the commercial process.

Download Word (.docx) Download PDF

4. Field reference

Every input in the tool, panel by panel. Tables accept rows pasted straight from Excel, comma-separated text, or a loaded file; check the columns after loading a file. Fields marked “when it applies” appear only for the choices that need them.

1 · Product and dose

FIELD	WHAT TO ENTER
Product	Type it in.
Submission region	Choose one: US: FDA (NDA, ANDA, BLA, IND); EU: EMA and national agencies (MAA); Both US and EU.
Specification for	Choose one: Drug substance (3.2.S.4); Drug product (3.2.P.5).
Route	Choose one: Intravenous; Subcutaneous; Intramuscular; Intrathecal.

FIELD	WHAT TO ENTER
Maximum dose (mg)	Type a number.
Shortest infusion time (h) when it applies	Shown for intravenous use; the endotoxin limit uses the dose per kg per hour. Type a number.
Protein concentration (mg/mL)	Type a number.

2 · Characterisation (3.2.S.3.1)

One row per attribute studied. The tool checks the ICH Q6B list: primary structure, PTMs, glycans, higher-order structure, variants and biological activity.

FIELD	WHAT TO ENTER
Characterisation results	A table. Columns: Attribute Method Result Lots tested. Paste rows from Excel, type comma-separated rows, or press Load file (Excel, CSV, Word, PDF or a scan).

3 · Impurities (3.2.S.3.2)

FIELD	WHAT TO ENTER
Host cell protein in drug substance (ng/mg, max of lots)	Type a number.
HCP assay and coverage	Type it in.
Residual host cell DNA (pg/mg, max of lots)	Type a number.
Leached Protein A (ng/mg, max of lots)	Type a number.
Other process-related impurities	A table. Columns: Impurity Level or clearance Basis for control. Paste rows from Excel, type comma-separated rows, or press Load file (Excel, CSV, Word, PDF or a scan).
Product-related impurities	A table. Columns: Impurity Level in lots Biological activity or safety. Paste rows from Excel, type comma-separated rows, or press Load file (Excel, CSV, Word, PDF or a scan).

4 · Specification (3.2.S.4.1 or 3.2.P.5.1)

FIELD	WHAT TO ENTER
Tests and acceptance criteria	A table. Columns: Test Method Acceptance criterion Release / shelf life. Paste rows from Excel, type comma-separated rows, or press Load file (Excel, CSV, Word, PDF or a scan).
Justification approach (3.2.S.4.5 / 3.2.P.5.6)	Type or paste text.
Lots supporting the criteria	Potency and monomer of each supporting lot, checked against the proposed criteria. A table. Columns: Lot Use (toxicology / clinical / PPQ) Potency (%) Monomer (%). Paste rows from Excel, type comma-separated rows, or press Load file (Excel, CSV, Word, PDF or a scan).

5 · Reference standards (3.2.S.5)

FIELD	WHAT TO ENTER
Primary reference standard	Type it in.
Working reference standard	Type it in.
Protocol for new reference standards and stability monitoring	Type it in.

5. Checks and alerts

Calculations and pass/fail outcomes always come from fixed rules, never from AI. The tool checks:

- Every ICH Q6B attribute group is characterised, each with a result, and the lots used are named.
- HCP reported (flagged above about 100 ng/mg), with the assay and coverage; residual DNA reported (flagged above 10 ng per dose); product-related impurities and aggregates addressed.
- Each test has a criterion and a method; the endotoxin criterion is not above the limit from the dose (critical) and is in EU/mg or EU/mL.
- Supporting lots meet the proposed potency and monomer criteria; at least three lots, including clinical or toxicology lots; a justification is given.
- Primary and working reference standards and a protocol for new standards; criteria that apply at shelf life.

How to resolve alerts

- **Critical** alerts block the download and must be fixed in the data. A failing result can instead be linked to an investigation: type the investigation reference and outcome and press **Record**.
- **Major** alerts block the download until you fix them or type a justification and press **Accept justification**.
- **Minor** alerts do not block anything. They are listed in the documents so the reviewer sees them.
- Some alerts have an **Apply fix** button that makes the change for you. **Reopen** undoes a justification.

Narratives

This module writes: Characterisation summary; Impurities; Justification of specification. Each narrative is written by fixed rules from your data. You can edit it in the box. **Rewrite with AI** (where your plan includes AI) rewrites it in regulatory style; every number in the text is checked against your data, and a number that does not match is flagged under the box and blocks the download until you correct it.

Reset to rule-based text brings back the original.

6. Preview, gap review and download

1. Press **Preview** next to a section, or **Preview and download all**. The document opens in a preview window, exactly as the Word file will read.
2. The left side lists the review, run on your device: **gaps in the data** (missing values, open alerts), **gaps in the report** (missing content the guidance expects, placeholders such as [site] or TBD left in the text) and, if you load the approved protocol under **Compare with the protocol**, **differences from the protocol** (requirements, criteria, batches or time points it plans that the report does not show).
3. Click a finding to jump to the place in the preview. Fix it in the tool, then preview again.
4. Optional **AI review**: a second read for contradictions, claims without data and vague wording. The report text is sent to Claude only when you press it, and only where your plan includes AI. Check every AI finding; AI can be wrong.
5. Press **Download Word (.docx)** or **Download PDF**. With several documents open, **All as Word (.zip)** and **All as PDF (.zip)** download the set. The download buttons stay disabled while a blocking alert is open; the preview still works.

Documents this module produces

SECTION	DOCUMENT
3.2.S.3.1	Elucidation of structure and other characteristics

SECTION	DOCUMENT
3.2.S.3.2	Impurities
3.2.P.5.1 / 3.2.S.4.1	Specification(s)
3.2.P.5.6 / 3.2.S.4.5	Justification of specification(s)
3.2.S.5	Reference standards or materials

Use **Save project** to keep your work as a file on your computer, and **Open project** to continue later. **Start blank** clears every field; **Load example** brings the example back. Nothing is stored on CMCfy servers.

7. US and EU submissions

US mode cites USP methods (for example USP <85> bacterial endotoxins, <787>/<788> particulate matter). EU mode expects Ph. Eur. methods (2.6.14, 2.9.19) and flags methods that cite USP/NF only; choose both regions to cite both. ICH Q6B applies in both.

8. Working with other modules

- Clearance of HCP, DNA and Protein A is shown in the Biologics Process Writer.
- Stability-indicating attributes and limits go to the Biologics Stability Studio module.
- For small molecules use the Specification Builder instead.

9. Common pitfalls

- Entering HCP in ng per dose instead of ng/mg: the per-dose calculation does that for you.
- Forgetting the infusion time for IV products, which changes the endotoxin limit.
- Setting criteria tighter than a clinical lot already released, or looser than lots support without justification.
- No working standard or no protocol for replacing standards.

10. Practice exercises

Each exercise starts from the example (press **Load example** to reset). We run these on every update of this guide, so the result you see should match.

1. Double the dose

Change **Maximum dose (mg)** from 1500 to 3000.

EXPECTED RESULT

The endotoxin limit from the dose in the headline note becomes 0.12 EU/mg.

2. Enter a high host cell protein level

Change **Host cell protein in drug substance** to 150.

EXPECTED RESULT

A major alert says HCP is 150 ng/mg, above the level usually seen for a purified product.

Guide updated 28 October 2026 for the current version of the tool. CMCfy drafts are for review by a qualified person before use in a submission.