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Cell Line Characterization and Product Testing for CHO-Derived Biopharmaceutical Products

Eurofins BioPharma Product Testing is a global leader in comprehensive laboratory services enabling pharmaceutical and biopharmaceutical companies to advance candidates from development through commercialization while ensuring regulatory compliance, cost effectiveness and achievement of timelines.

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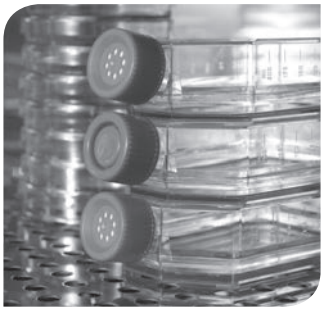

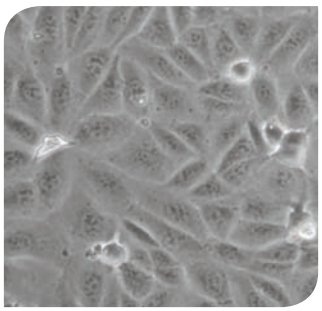

A global leader in biopharmaceutical laboratory services, Eurofins BioPharma Product Testing provides you with the tools and support to better manage your biopharmaceutical development process. Our vast capacity and extensive capabilities provide the greatest breadth of cGMP-compliant biopharmaceutical services in the industry.

Eurofins BioPharma Product Testing offers capabilities to prepare and characterize a wide variety of mammalian, insect and avian cell banks, including master, working and research banks. Our cGMP-compliant facilities include multiple Grade A/B suites and ISO 7 clean rooms with ISO 5 critical areas designed to meet current FDA aseptic processing guidelines.

We also offer a comprehensive package of safety testing services in the areas of microbiology, mycoplasma, genetic stability, viral safety and biochemistry to support our cell banking capabilities. All of these services are provided with strict adherence to cGMP requirements and are designed to fully support the testing of your biopharmaceutical product, including the testing of raw materials, cell lines, unprocessed bulk, purified bulk and final product.

This document provides Eurofins BioPharma Product Testing's recommendations for cell line characterization, safety evaluation and product testing for a CHO-derived biopharmaceutical product. It outlines a testing strategy that should satisfy the latest guidance of global regulatory authorities, including the FDA and EMA. However, it may not be necessary to conduct all of the tests listed. We highly recommend a discussion to evaluate those factors that can influence which tests can be omitted or whether additional testing might be required based on our experience.

The combination of our manufacturing capabilities with our extensive characterization services provide you with a single-source solution for all of your cell line and product testing needs.

Cell Line Characterization and Product Testing for CHO-Derived Biopharmaceutical Products

Cell Seed Testing					
Test	Identity [1]	Genetic Stability	Sterility [2]	Mycoplasma	Viruses[1]
Bacteriostasis/Fungistasis by Direct Transfer			X		
Sterility Testing - Non Isolator			X		
Mycoplasmastasis				X	
Mycoplasma (rapid method also offered)				X	

[1] Additional testing may be advised after a technical discussion. For example, identity testing may be performed to ensure the cells are the correct species before production of the MCB. Limited viral screening may also be completed during this phase to mitigate risk.
[2] Container ingress studies may be required prior to performance of the sterility test in isolators. Alternatively, a non-isolator sterility test may be performed.

Master Cell Bank Testing					
Test	Identity	Genetic Stability [1]	Sterility	Mycoplasma	Viruses
CO1/Cyt B Sizing or CO1 Barcoding	X				
Copy Number by qPCR		X			
Restriction Mapping by Southern Blot		X			
DNA Sequencing		X			
Bacteriostasis/Fungistasis by Direct Transfer [2]			X		
Sterility by Direct Transfer			X		
Mycoplasmastasis [2]				X	
Mycoplasma (rapid method also offered)				X	
In Vitro Adventitious Viruses (28 day test, 3 cell lines) [3]					X
In Vitro Adventitious Viruses for MMV (28 day test, 1 cell line) [3]					X
In Vivo Adventitious Viruses [8]					X
Transmission Electron Microscopy [4]					X
Reverse Transcriptase Assay [3, 5]					X
S^+L^- Focus Forming Assay for Xenotropic Retroviruses [3]					X
Hamster Antibody Production (HAP) [8]					X
Mouse Antibody Production (MAP) [8]					X
Detection of MMV DNA by qPCR [9]					X
Porcine Viruses by 9CFR [3, 6]					X
Bovine Viruses 9CFR [3, 7]					X

[1] A technical discussion is required to determine an appropriate approach to genetic stability testing.
[2] Bacteriostasis/fungistasis and mycoplasmastasis may not be required if the results from seed cell testing are applicable to the MCB.
[3] A technical discussion is recommended to discuss matrix interference testing for this assay.
[4] This assay is subcontracted to an approved vendor and performed as a GLP assay.
[5] Reverse Transcriptase Testing should be discussed further as a positive result is not unexpected with CHO cells.
[6] Porcine 9CFR testing is advised if the cells have ever been in contact with porcine trypsin or any other porcine-derived product.
[7] Bovine 9CFR testing is advised if the cells have ever been in contact with bovine serum or any other bovine-derived product.
[8] This assay is subcontracted to an approved vendor.
[9] A technical discussion is recommended to discuss matrix interference testing for this assay. Other viral qPCR assays are available upon request.

Cell Line Characterization and Product Testing for CHO-Derived Biopharmaceutical Products

Working Cell Bank Testing					
Test	Identity	Genetic Stability	Sterility	Mycoplasma	Viruses
CO1/Cyt B Sizing or CO1 Barcoding	X				
Bacteriostasis/Fungistasis by Direct Transfer [1]			X		
Sterility by Direct Transfer			X		
Mycoplasmastasis [1]				X	
Mycoplasma (rapid method also offered)				X	
In Vitro Adventitious Viruses (14 or 28 day test, 3 cell lines) [2]					X
In Vitro Adventitious Viruses for MMV (28 day test, 1 cell line) [2]					X
In Vivo Adventitious Viruses [3]					X

[1] Bacteriostasis/fungistasis and mycoplasmastasis may not be required if the results from seed cell and/or MCB testing are applicable to the WCB.
[2] A technical discussion is recommended to discuss matrix interference testing for this assay.
[3] *In vivo* adventitious test is not required if this testing will be done on EOP cells. Also see footnote 3 under unprocessed bulk testing. This assay is subcontracted to an approved vendor.

End of Production Cell Bank Testing (Cells at Limit of *In-Vitro* Age)					
Test	Identity	Genetic Stability [1]	Sterility	Mycoplasma	Viruses
CO1/Cyt B Sizing or CO1 Barcoding	X				
Copy Number by qPCR		X			
Restriction Mapping by Southern Blot		X			
DNA Sequencing		X			
Bacteriostasis/Fungistasis by Direct Transfer			X		
Sterility Testing - Non Isolator			X		
Mycoplasmastasis				X	
Mycoplasma (rapid method also offered)				X	
In Vitro Adventitious Viruses (28 day test, 3 cell lines) [2]					X
In Vitro Adventitious Viruses for MMV (28 day test, 1 cell line) [2]					X
In Vivo Adventitious Viruses [3]					X
Transmission Electron Microscopy [3]					X
Reverse Transcriptase Assay [2, 4]					X
S^+L^- Focus Forming Assay for Xenotropic Retroviruses [2]					X
Hamster Antibody Production (HAP) [3]					X
Mouse Antibody Production (MAP) [3]					X
Detection of MMV DNA by qPCR [7]					X
Porcine Viruses by 9CFR [2, 5]					X
Bovine Viruses by 9CFR [2, 6]					X

[1] A technical discussion is required to determine an appropriate approach to genetic stability testing.
[2] A technical discussion is recommended to discuss matrix interference testing for this assay.
[3] This assay is subcontracted to an approved vendor and performed as a GLP assay.
[4] Reverse Transcriptase Testing should be discussed in more detail as a positive result is not unexpected with CHO cells.
[5] Porcine 9CFR testing is advised if the cells have ever been in contact with porcine trypsin or any other porcine-derived product.
[6] Bovine 9CFR testing is advised if the cells have ever been in contact with bovine serum or any other bovine-derived product.
[7] A technical discussion is recommended to discuss matrix interference testing for this assay. Other viral qPCR assays are available upon request.

Cell Line Characterization and Product Testing for CHO-Derived Biopharmaceutical Products

Unprocessed Bulk Testing					
Test	Identity	Genetic Stability	Sterility	Mycoplasma	Viruses
Bacteriostasis/Fungistasis by Direct Transfer			X		
Sterility by Direct Transfer [1]			X		
Mycoplasmastasis				X	
Mycoplasma (rapid method also offered)				X	
In Vitro Adventitious Viruses (28 day test, 3 cell lines) [2]					X
In Vitro Adventitious Viruses for MMV (28 day test, 1 cell line) [2]					X
In Vivo Adventitious Viruses [3]					X
Transmission Electron Microscopy [4]					X
Detection of MMV DNA by qPCR [5]					X

[1] Testing for bioburden may be more appropriate than sterility testing.
[2] A technical discussion is recommended to discuss matrix interference testing for this assay. Other viral qPCR assays available upon request.
[3] *In vivo* testing of the unprocessed bulk is unusual but may be requested by a regulator as one-time testing or after a change in bioreactor conditions has been made. *In vivo* testing is also performed if EOP cells are not tested during early stages of product development. This assay is subcontracted to an approved vendor.
[4] This assay is performed as a GLP assay and is typically performed only on the first three consecutive lots.
[5] A technical discussion is recommended to discuss matrix interference testing for this assay. Other viral qPCR assays available upon request.

Purified Bulk & Final Product Testing								
Test	Identity	Genetic Stability	Sterility	Mycoplasma	Viruses	General Safety	Residual Impurity	Analytical Assays[3]
Bacteriostasis/Fungistasis			X					
Sterility by Direct Transfer			X					
Bacterial Endotoxin [1]			X					
General Safety Test [2]						X		
Residual CHO DNA by qPCR							X	
CHO Host Cell Protein by ELISA							X	
Protein A by ELISA							X	
Amino Acid Analysis								X
Appearance of Solution								X
Biopotency Assay								X
Carbohydrate Mapping								X
Monosaccharide Analysis								X
Protein Content Assay								X
Analysis by HPLC								X
Moisture by Karl Fischer								X
N-Terminal Sequencing								X
Osmolality								X
Peptide Mapping								X
pH Determination								X
SDS-PAGE								X
UV Absorbance								X
Western Blot								X

[1] Endotoxin testing is for final product only.
[2] This assay is subcontracted to an approved vendor.
[3] A technical discussion with regard to analytical testing is advised. The tests listed are examples of the types of tests offered and which are often requested by clients.

Cell Line Characterization and Product Testing for CHO-Derived Biopharmaceutical Products

Test	TAT*	Sample Requirements	Storage
Amino Acid Analysis	10 business days	>10 mg powder	-20°C or below
Analysis by HPLC (ion exchange separation)	10 business days	>1 mg	-20°C or below
Analysis by HPLC (reverse phase separation)	10 business days	>1 mg	-20°C or below
Analysis by HPLC (size exclusion separation)	10 business days	>1 mg	2° to 8°C
Appearance of Solution	10 business days	variable	variable
Bacterial Endotoxin - Inhibition/Enhancement	10 business days	2-5 mL	variable
Bacterial Endotoxin	10 business days	2-5 mL	variable
Bacteriostasis/Fungistasis	17 business days	variable	variable
Biopotency Assay	3 weeks	0.5-1 mL if in liquid form, 25 mg solid API	variable
Bovine Viruses	5 weeks	8 mL cell lysate at 1E7 cells/mL	-60°C or below
Carbohydrate Mapping	10 business days	>1 mg	-20°C or below
CHO Host Cell Protein by ELISA	2 weeks	2 mL if in liquid form, 25 mg solid API	-60°C or below
Copy Number by qPCR	3 weeks	2 x 1E7 cell pellet	-60°C or below
Detection of MMV DNA by qPCR	2 weeks	4 mL for matrix qualification, 2 mL for routine	-20°C or below
DNA Sequencing	Custom	2E7 cell pellet if nonbacterial, 1 glycerol stock or 1E8 cell pellet if bacterial	-60°C or below
General Safety Test	4 weeks	15 mL	variable
Hamster Antibody Production (HAP)	7 weeks	5 mL cell lysate at 1E7 cells/mL	-60°C or below
CO1/Cyt B Sizing	10 business days	2E7 cells	-60°C or below
CO1 Barcoding	15 business days	2E7 cells	-60°C or below
In Vivo Adventitious Viruses	7 Weeks	32+8+3+2X2 mL bulk harvest or cell lysate at 1E7 cells/mL	-60°C or below
In Vitro Adventitious Viruses (28 day test, 3 cell lines)	6 weeks	20 mL bulk harvest or cell lysate at 1E7 cells/mL	-60°C or below
In Vitro Adventitious Viruses for MMV (28 day test, 1 cell line)	6 weeks	7 mL bulk harvest or cell lysate at 1E7 cells/mL	-60°C or below
Moisture by Karl Fischer	2 weeks	variable	variable
Monosaccharide Analysis	10 business days	>1 mg	-20°C or below
Mouse Antibody Production (MAP)	7 weeks	5 mL cell lysate at 1E7 cells/mL	-60°C or below
Mycoplasma (Cells)	25 business days	2 x 12 mL, 2 x 1.5 mL $\geq$ 1 x 10^6 cells/mL	-60°C without cryoprotectant
Mycoplasmastasis (Cells)	20 business days	2 x 25 mL, 4 x 1.5 mL $\geq$ 1 x 10^6 cells/mL	-60°C without cryoprotectant
Mycoplasma (Unprocessed Bulk)	25 business days	2 x 12 mL, 2 x 1.5 mL	-60°C
Mycoplasmastasis (Unprocessed Bulk)	20 business days	2 x 25 mL, 4 x 1.5 mL	-60°C
N-Terminal Sequencing	10 business days	0.5 mg protein	-20°C or below
Oligosaccharide Profile (Glycan Analysis)	10 business days	0.5 mg protein	-20°C or below
Osmolality	10 business days	variable	-20°C or below
Peptide Mapping	10 business days	>1 mg	-20°C or below
pH Determination	10 business days	variable	-20°C or below
Porcine Viruses	5 weeks	10 mL cell lysate at 1E7 cells/ml	-60°C or below
Protein A by ELISA	2 Weeks	2 mL if in liquid form, 25 mg solid API	-60°C or below
Protein Content Assay	10 business days	dependent on extinction coefficient	-20°C or below
Residual CHO DNA by qPCR	2 weeks	4 mL for matrix qualification, 2 mL for routine	-20°C or below
Restriction Mapping by Southern Blot	6-8 weeks	2E7 - 4E7 cell pellet	-60°C or below
Reverse Transcriptase Assay	3 weeks	2 mL supernatant for matrix qualification, 2 mL routine	-60°C or below
S^+L^- Focus Forming Assay for Xenotropic Retroviruses	5 weeks	5 mL culture supernatant	-60°C or below
SDS-PAGE	10 business days	0.5 mg protein	-20°C or below
Sterility	17 business days	variable	variable
Transmission Electron Microscopy (Cells)	10-12 weeks	fixed cell pellet, at least 2E7 cells	2° to 8°C
Transmission Electron Microscopy (Supernatant)	10-12 weeks	10 mL bioreactor harvest	-60°C or below
UV Absorbance	10 business days	dependent on ext. coeff.	-20°C or below
Western Blot	10 business days	0.5mg protein	-20°C or below

*Standard turnaround time. Expedited turnaround may be available with prior notification.

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