

Allegro™ XRS 25 Bioreactor System



Unique bi-axial agitation improves cell culture performance

The Allegro XRS 25 bioreactor system is a single-use bioreactor system with unique agitation and control properties designed for the cultivation of mammalian cells in suspension culture under controlled conditions. It is suitable for applications ranging from general life sciences research to seed train operations and full cGMP production at the 2 to 25 liter scale.

Because of its unique bi-axial agitation and the subsequent shorter mixing times and higher mass transfer properties, the Allegro XRS 25 bioreactor system produces superior performance compared to competitor rocker systems, as measured by higher density, better cell viability, and higher expression levels.

The Allegro XRS 25 bioreactor system rocks on two axes simultaneously (bi-axial agitation), producing a low turbulence, swirling pattern within the 3-dimensional XRS 25 biocontainer. Under similar agitation conditions, the Allegro XRS 25 bioreactor system mixes almost 3 times faster than conventional rockers (Table 1), and demonstrates much higher mass transfer properties as measured by k_La values (Table 2).

For more information:

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Table 1

Summary of best mixing times observed in pH equilibration studies, 20 liter working volumes with conventional pH probes¹

System	Condition	Mixing Time(s)
Conventional rocker	Maximum speed and rock angle: 42 rpm, 10.4 °	50 to 98 seconds ²
Allegro XRS 25 bioreactor system	75% maximum speed, maximum long axis angle, 1/3 short axis angle: 30 rpm, 15 ° long, 5 ° short	16 seconds

Table 2

Summary of mass transfer coefficients observed in 20 liter working volumes as measured with nitrogen purge and recovery³

System	Condition	k _L a per Hour
Conventional rocker	Maximum speed and angle: 42 rpm, 10.4 °	42
Allegro XRS 25 bioreactor system	40 rpm, 15 ° long, 15 ° short	73

¹ Mixing trial results from three repetitions for each device.

² Times significantly different for edge/center measurements.

³ Mass transfer results from three repetitions for each device.

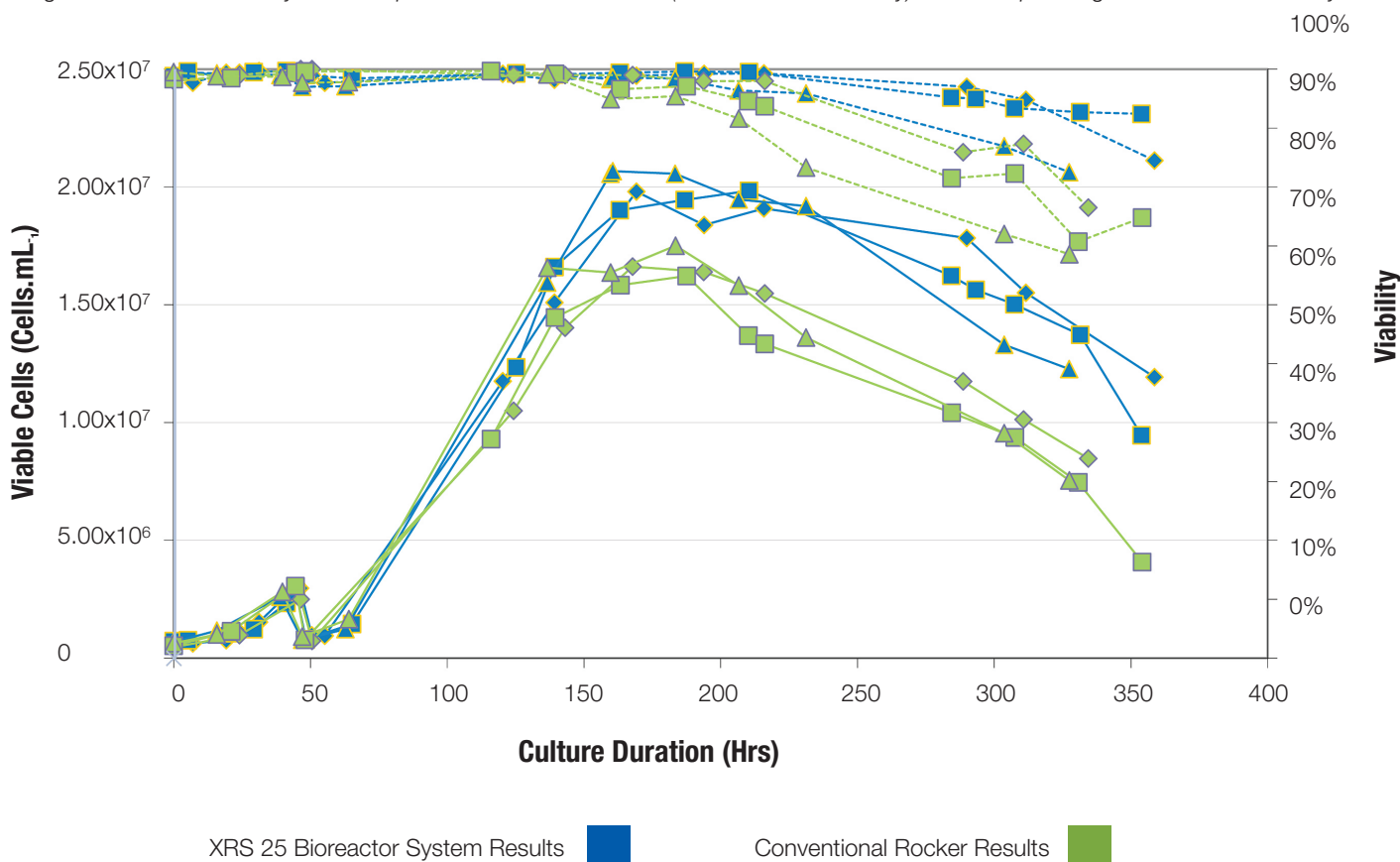
For additional details on mixing and mass transfer studies, see Pall application note USD 2899: Performance Mapping in the Pall XRS 20 Bioreactor System.

Applications

The mixing properties of the 3D biocontainer design and bi-axial agitation lead to superior cell culture performance in mammalian suspension systems. With faster delivery of nutrients to the cellular micro-environment (and faster removal of metabolites), the Allegro XRS 25 bioreactor system allows cells to attain higher densities, with higher overall viabilities, leading to significantly higher protein expression levels. In the batch culture example below (Figure 1), with identical media inputs and seeds, the Allegro XRS 25 bioreactor system produced 18% higher cell densities, and 30% more antibody titer compared to conventional rocker technologies.

Figure 1

Summary of side-by-side growth and viability experiments (n=3) at 20 liter working volumes comparing conventional rocker system and Allegro XRS 25 bioreactor system. Simple batch culture of a CHO (Chinese hamster ovary) cell line expressing a monoclonal antibody.



The excellent mixing properties of the Allegro XRS 25 bioreactor system have also shown to be beneficial for growing batch, fed batch and perfusion cultures of mammalian (CHO, hybridoma) and insect cells.

System Overview, Features and Benefits

Figure 2

Allegro XRS 25 bioreactor system – featuring the agitation platform, the single-use biocontainer and the mPath™ control tower with Pall Link supervisory control and data acquisition (SCADA) software package. The Allegro XRS 25 bioreactor system incorporates many features to ensure easy operation, robust performance, and suitability for cGMP operations.



Table 3

Allegro XRS 25 Biocontainer

Feature	Benefit
3 dimensional biocontainer design	▶ Superior mixing (with bi-axial platform), robust in operation
Biocontainer with integrated optical sensors, integrated filters, pre-assembled tubing sets	▶ True single-use system, ease of installation, minimal turn-around time
Gas inlet and exhaust filters are integrity testable, sterilizing grade 0.2 µm	▶ Improved suitability for cGMP operations
Integrated bottom drain with rotary lift valve	▶ Improved suitability for cGMP operations, minimal hold-up volume, ease of use
Multiple liquid addition ports	▶ Improved ease of use, easily handles fed batch operations, acid/base pH control feeds
Integrated single-use pH and dissolved oxygen (DO) sensors	▶ Ease of use and reduced probability of contamination
Integrated sampling port	▶ Ease of use – very low hold up volume (less than 5 mL), capable of sampling without stopping agitation
Single-use biocontainer built with Allegro film	▶ Assurance of robustness, quality and supply, track record of use of film in cGMP operations, validation guide available

Table 4

Allegro XRS 25 Platform

Feature	Benefit
Bi-axial agitation (rocking motion on two horizontal axes at 90 ° separation)	▶ Improved agitation properties: shorter mixing times and higher mass transfer rates
Fully enclosed platform with safety interlock	▶ Improved operator safety, suitable for production environments, protects light sensitive culture media

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Table 5

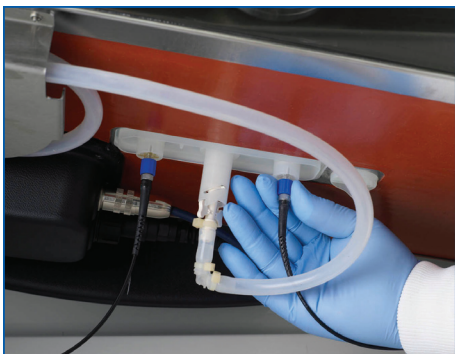
mPath Control Tower and Pall Link Server

Feature	Benefit
mPath control tower designed for operation with Pall Link – a SCADA software package	▶ Capable of accessing data anywhere on any networked device including laboratory workstations, desktop computers or remote devices such as laptops, mobile phones and tablets ▶ Operates as either a standalone network or integrated with site IT systems, compatible with multiple network security approaches (e.g. virtual private network [VPN]) and password protection to protect bioreactors from unauthorized access ▶ Intuitive visual displays for easy interaction with bioreactors to calibrate components, set critical parameters, and fine tune process control ▶ Advanced trending capability including creation of custom trends of any process variable for both historic and live processes ▶ Database centric design supports naming, logging and exporting experiments for traceability and data analysis ▶ Full alarm customization to alert for process or safety critical events ▶ Management features for user-definable recipes including scheduling, version control and retirement for recipes no longer in use ▶ Compliance capability – designed for operation following 21 CFR Part 11/ Annex 11 regulations
Multiple alarm notification modalities to ensure response to process critical events	▶ Color coded illumination ring around the front face of the control tower allows identification of operating state from a distance ▶ Individual LED indicators for each connection on the connector panel identify problems during set up and operation ▶ Highly visible color and textual alerts indicate alarm severity on all software interfaces ▶ Historical alarm logs to review equipment and process performance

Table 5 *continued*

Feature	Benefit
Gas handling by six oxygen-rated thermal mass flow controllers (TMFCs) pre-calibrated for process gases	▶ Accurate gas mixing for two gas streams enabling accurate parameter control and complex gas control regimes, each gas within the mix delivered by a dedicated TMFC ▶ Primary gas mix: N₂, O₂, air and CO₂ ▶ Secondary gas mix: CO₂ and air ▶ Calibrated flow rates up to 1 L/min for each TMFC ▶ Onboard regulators ensure accuracy of TMFCs even with higher input pressures ▶ Onboard pressure sensors, combined with alarming capability enable notification of empty gas cylinders or biocontainer overpressure ▶ Fast fill button – quick and safe inflation of biocontainers with air
Liquid handling with three variable speed pumps, integrated load cells and biocontainer hangers	▶ Accurate delivery of feeds and titrants without compromises, standard variable speed pumps enable smoother process control and feed profiles for less process variability ▶ 8 mm max tubing outside diameter (OD) ▶ 14 - 211 rpm (control requiring speeds less than 14 rpm achieved through pulse width modulation [PWM]) ▶ Removable pump heads for easy cleaning and serviceability ▶ Removable biocontainer hangers with integrated load cells allow space efficient execution of weight based feed profiles and accurate tracking of titrant delivery
Ergonomic industrial design	▶ Extensive human factors research to determine optimal placement of connections and control tower elements ▶ Flexibility in operation – choose when and where to view data, choose how to design processes ▶ Compact design to limit bench space requirements – small form factor and biocontainer hangers remove the need for scales and bottles ▶ Elevated from bench for cleanliness

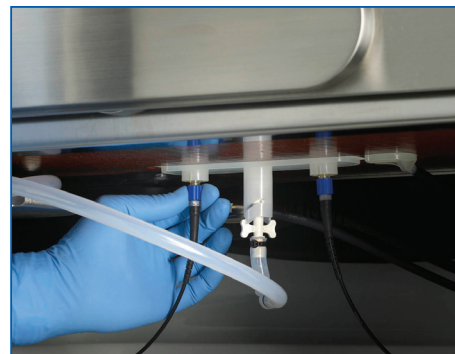
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Tubing management system



In-process sampling



Drain and optical sensors

Customization

Pall has standard Allegro XRS 25 biocontainers equipped with all liquid and gas connections for the most typical processes and most operations. The three feed lines, the accessory line, and the drain are fitted with standard laboratory connections. Process liquids, including media and inoculum, can easily and safely be transferred into the bioreactor.

Pall can assist in customizing the bioreactor systems. This includes adding aseptic connectors (Kleenpak® sterile connectors), and small changes on the biocontainer up to and including integrating the bioreactor with all surrounding unit operations into a fully automated process.

Documentation and Certificates

The Allegro XRS bioreactor is delivered with complete documentation that qualifies the system. All units are tested prior to shipment and witnessed Factory Acceptance Testing (FAT) is available on request.

The biocontainers are delivered with all the batch specific certificates and calibration information for the included single-use sensors.

Pall also can assist with hardware qualification, and standard installation verification (IV), operational verification (OV), factory acceptance test (FAT) and site acceptance test (SAT) test protocols are available.



Integrated drain simplifies harvest operations

Pall Quality Standards

Pall maintains a very stringent approach to quality of purchased and manufactured components. Allegro bioreactor systems are designed and built to well recognized industry standards. The Allegro XRS 25 bioreactor system hardware meets many quality standards including:

- ▶ Good automated manufacturing practice (GAMP) current version
- ▶ 21 CFR Part 11 for electronic records
- ▶ Electromagnetic compatibility (EMC) to EN 61326-1: 2013 and FCC CFR 47 Part 15B: 2013
- ▶ The product is made in a manufacturing facility where the quality management system is ISO9001: 2008 certified.

A full list of quality standards is available on request

The Allegro XRS 25 biocontainer quality standards include:

- ▶ The product is made in a manufacturing facility where the quality management system is ISO13485 and ISO9001 certified
- ▶ 100% of Allegro XRS 25 biocontainers are checked for correct components, configuration, welding standard and dimensional correctness. A visual inspection confirms there are no embedded particles in the film or any loose particles inside or outside of the biocontainer
- ▶ 100% of Allegro XRS 25 biocontainer chambers undergo a pressure leak test during manufacturing
- ▶ Samples from each biocontainer manufacturing batch undergo a tensile test of the film weld area and a microscope inspection of the weld area to ensure the quality of the welded seal
- ▶ Fluid path endotoxins: periodically, rinse effluents from representative samples of Allegro systems are tested for endotoxins in accordance with USP <85> using limulus amoebocyte lysate (LAL) reagent. Fluid path rinses meet the internal specification of < 0.25 EU/mL
- ▶ Fluid path cleanliness: periodically, rinse effluents from representative samples of Allegro systems are tested for particulates. Fluid path rinses meet the current limits under USP <788> particulate matter in injections

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The materials of construction of the Allegro XRS 25 biocontainer meet:

- ▶ USP<88> Biological reactivity test *in vivo* for class VI - 50 °C plastics
- ▶ USP<87> Biological reactivity tests *in vivo*, cytotoxicity
- ▶ USP<85> Bacterial endotoxin tests
- ▶ USP<661> Physico-chemical testing for plastics
- ▶ USP<788> Particulate matter in injections

Materials of construction for all components in contact with fluid are TSE/BSE risk materials free.

All Allegro XRS 25 biocontainers are supplied double bagged (outer layer opaque to protect the single-use sensors from light) and irradiated at a minimum dose of 25 kGy.



Preventive Maintenance and Service Packages

The Allegro single-use bioreactor systems are supported with extensive maintenance and service packages. Pall has a global network of technicians and engineers available to assist with installation, qualification, training, technical support, preventive maintenance and on-site repairs. Contact Pall to arrange to discuss any of these services.

Pall Accelerator process development services provide customers with expert advice and support for the implementation of fully integrated single-use processing solutions into new or existing processes, including process optimization, equipment selection, transfer of existing processes, scale-up and troubleshooting advice. Details can be found in the Accelerator Process Development Services brochure (USD 3079).

Preventive maintenance packages are available to ensure the continuous reliable operation of the Allegro XRS 25 bioreactor and to minimize downtime. Preventive maintenance typically includes a full functional test, exchange of wear parts, and calibration of all sensors.

General System Specifications

Table 6

Dimensions (W x D x H)	Controller	600 mm x 450 mm x 230 mm
	Platform	664 mm x 379 mm x 331 (allow for 200 mm lid overhang at the rear of the platform, and factor in an additional 370 mm for height when the lid is open)
Weight	Controller	20 kg
	Platform (with filled biocontainer)	78 kg (104 kg filled)
Gases	Gas connections	Inlet and outlet quick connectors 6 mm OD
	Gas flow control	TMFC
	TMFC pressurized air	0 - 1000 mL/min
	TMFC oxygen	0 - 1000 mL/min
	TMFC carbon dioxide	0 - 1000 mL/min
Pumps	Quantity and connection	3 x variable speed peristaltic pumps, 0 - 211 rpm, maximum tubing OD 8 mm
Aeration	Inlet filter	0.2 µm single-use filter
	Gas delivery	Gas mix injected into biocontainer headspace
	Exhaust filter	0.2 µm single-use filter
	Exhaust heater	Silicone heater mat
Bi-Axial Agitation	Speed	1 - 35 rpm
	Angle	-15 to +15 degrees in both X and Y directions
Process	Control	Stepper motor controlled
	Working volumes	2 - 25 liters
	Temperature control method	Heating blanket, ambient convective cooling
	Temperature range	10 - 40 °C
	pH control	6 - 8, integrated single-use optical sensor
	Dissolved oxygen control	0 - 100%, integrated single-use optical sensor

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The Allegro XRS 25 bioreactor system is available as a standard product; however, both the bioreactor hardware and consumables can be customized. Our teams are specialized in upstream and downstream processing and will gladly help find the right technology for any part of your manufacturing process. It is possible to combine the Allegro products to meet any requirement in the upstream part of the process:

- ▶ Media preparation
- ▶ Buffer preparation
- ▶ Media sterilization and aseptic transfer
- ▶ Liquid transfer into the bioreactor (e.g. glucose, anti-foam, base, etc.)
- ▶ Seed train solutions
- ▶ Cell harvest and separation
- ▶ Perfusion modules

Please contact Pall for a total solution discussion on your process.



Allegro XRS 25 bioreactor system integrated into total upstream processing solution

Ordering Information

Table 7

Description	Part Number
Allegro XRS 25 Bioreactor System:	XRS25BRS
System hardware – consists of Allegro XRS 25 platform, mPath controller, control cables	
Pall Link server and SCADA software	MPATHLINK
Standard Allegro XRS 25 biocontainer	609-40A
Accessory Manifolds:	
Kleenpak™ manifold with vent filter	609-100A
Luer to Y connection and Kleenpak connectors	629-100B
MPC to Y connection and Kleenpak connectors	629-100C
5 liter 2D Allegro accessory biocontainer with filling filter, Kleenpak connector	609-100D
1 liter 2D Allegro accessory biocontainer with filling filter, Kleenpak connector	609-100E
20 liter 2D Allegro accessory biocontainer with filling filter, Kleenpak connector	609-100F

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