



Allegro™ STR Single-Use Stirred Tank Bioreactors



Redefining single-use cell culture performance

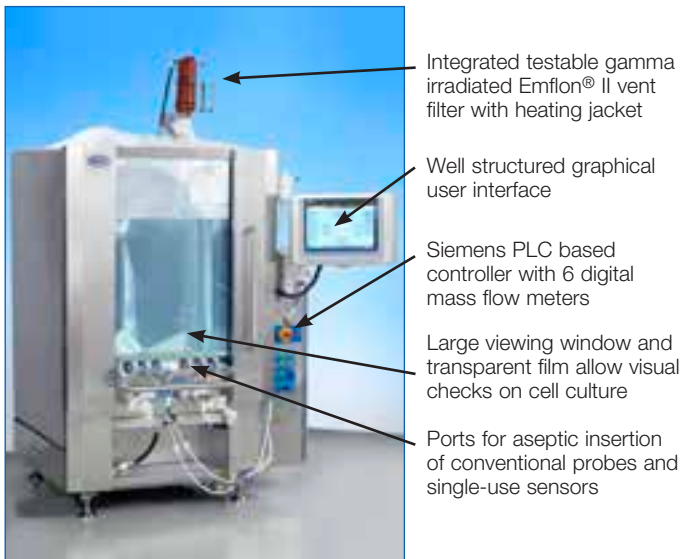
The Allegro STR bioreactor family combines Pall's bioprocess engineering expertise, cell culture know-how and our drive for quality into a series of single-use bioreactors that deliver consistent and scalable cell culture performance across the range. The Allegro STR bioreactor family has a direct bottom-driven impellor allowing power inputs up to 0.25 W/kg, resulting in high oxygen transfer rates and short mixing times.

From the outset of the design, our technology placed strong emphasis toward providing a compact, ergonomic and intuitive turn key bioreactor design concept to maximize usability and process assurance, while maintaining optimal performance needed in a cell culture environment through several easy and intuitive operation features:

- ▶ The single bottom-mounted impeller design allows the biocontainer to be packed into a compact unit that can be placed directly into the hardware with minimal operator manipulation
- ▶ Installation and inflation of the biocontainer is achieved in < 30 minutes through a guided sequence via the Human Machine Interface (HMI)
- ▶ The low aspect ratio of the systems minimize the operations that have to be performed at height
- ▶ Automatic biocontainer deflation speeds up turnaround time, minimizes operator activity and reduces the overall volume of waste for disposal.

All product contact surfaces in the Allegro STR bioreactors are single-use components therefore the demands on maintenance, cleaning and cleaning validation are minimal. This makes single-use bioreactors the first choice for process development, CMO plants or multiproduct manufacturing facilities where maximum flexibility is required.

Extensive experiments were performed to characterize the bioreactor and gather data on mixing, mass transfer and cell culture performance. Additionally, all components of this bioreactor were rigorously tested, and several long term experiments were completed in order to ensure the integrity and compatibility of the materials with regards to cell culture demands. During the product development, 45 day life tests were performed under maximum operating conditions to prove the design of the single-use system: no leaks were observed during this testing. A smaller number of life tests were also performed for >100 days continuous operation with no leaks observed.



Features and Benefits

Performance

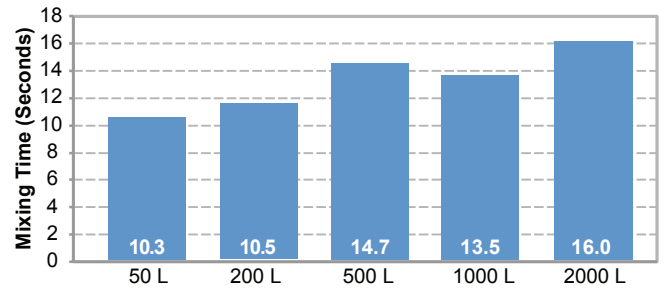
Mixing and k_La Performance:

► High k_La (up to 40 h^{-1}) and short mixing times (< 30 seconds) achieved by bottom mounted direct drive, pitched blade impeller.

► Typical k_La data for the Allegro STR bioreactor range:

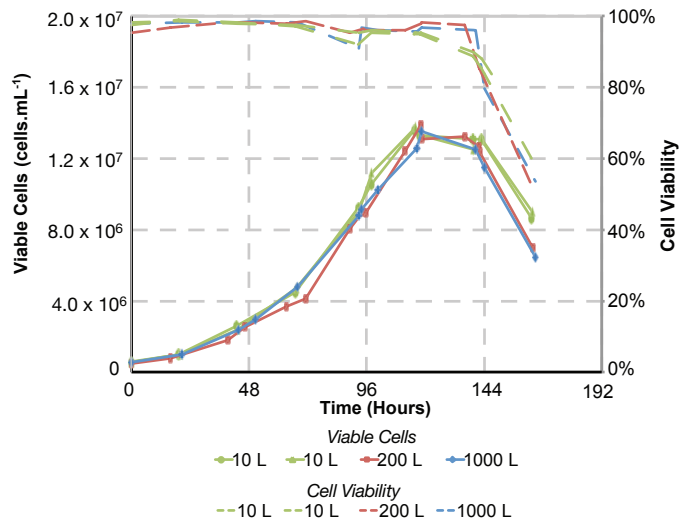
Allegro STR Scale (L)	Agitation Speed Range (rpm)	k_La Range (h^{-1})
50	25 – 200	0.31 – 24.2
200	25 – 150	2 – 33
500	25 – 150	0.05 – 20.5
1000	25 – 105	4 – 41
2000	25 – 105	7.5 – 29.5

► Typical mixing data for the Allegro STR bioreactor range:



► Mixing and k_La data demonstrate that the systems offer a broad design space allowing for good cell culture performance for a wide range of mammalian cell culture processes.

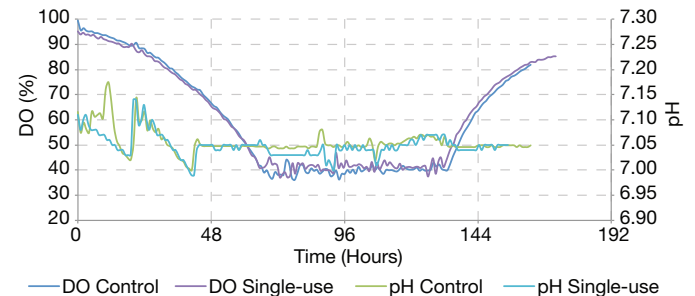
Cell Culture Performance and Scalability:



Please refer to Pall document USD3135 'Cultivation of Chinese Hamster Ovary (CHO) Cells in Allegro STR 1000 Single-Use Stirred Tank Bioreactor System' for further details.

► Geometric similarity across the product range ensures scalable performance across the Allegro STR bioreactor family

► Single-use pH and DO sensors provide equivalent performance to conventional (control) sensors



Usability

Simple Installation and Inflation

- ▶ Biocontainer easy to unpack and carry up to 2000 L scale
- ▶ Folded biocontainer easily placed directly onto floor of bioreactor hardware, no operator intervention to the film during installation
- ▶ Step-by-step instructions and color-coded numbered labels guide the operator through the processes
- ▶ Multi-point biocontainer directional arrows to ensure correct installation, and mitigate operator error during installation
- ▶ Installation and inflation of the biocontainer in less than 30 minutes
- ▶ Only one operator for most manipulations
- ▶ Automatic inflation sequence is enabled through self guided lifting arm¹ and preconfigured tubing management system
- ▶ Packaging unique rip cord design in packaging design to alleviate need for sharps being used setup and installation



Intuitive Operation

- ▶ Totally Integrated Siemens Automation Portal (TIA Portal) software platform with standard TFT color touch screen Panel PC (SIMATIC IPC 477D) enables ease of navigation while configuring setup or running an operation
- ▶ Easy to set up and run local control time based pump profiling to support various feeding strategies
- ▶ Alarms and status clearly identified with full audit trail available in a batch report
- ▶ Easy to establish different local cascades to maintain bioreactor control strategies to support culture demands
- ▶ Dynamic processing flexibility provided through either weight or time profile feeding features
- ▶ Clear to user when operator intervention is required



Working at Height Minimized

- ▶ Entire portfolio maintains low aspect ratio to reduce overall system height and increase efficiency on CO₂ strip rates
- ▶ Easy access from floor level to entire bioreactor at all volume sizes
- ▶ All Allegro STR bioreactors, including the 2000 L, height < 3 m
- ▶ No scaffolding required to operate when working at top of bioreactor



Process Assurance and Reliability

Biocontainer Packaging

- ▶ Compact and protective packaging
- ▶ Integral components provide support for biocontainer film, tubing and connectors during both installation and use
- ▶ Protects the biocontainer and components during transportation and installation



Allegro STR 1000 bioreactor

Allegro STR 50 bioreactor

Installation and Inflation

- ▶ Procedures predominantly automated
- ▶ Bioreactor microswitches provide operator feedback of correct step completion
- ▶ Minimizes operator intervention and prevents accidental damage
- ▶ Reduced risk for out of box failures
- ▶ Operator cannot advance to inflation sequence until correct installation is confirmed by the HMI (controlled via interlocks and switches on the hardware)



Sensors

- ▶ Can be operated with single-use sensors (pH and DO) and / or up to six conventional probes
- ▶ Additional analog connections for additional sensor integration (one sensor connection is configured for dissolved carbon dioxide probe input)
- ▶ High flexibility in process measurements
- ▶ Sensor calibration includes incorporated guided prompt sequence to ensure calibration is performed for batch consistency
- ▶ Allows integration of various process analytical measurements for process automation
- ▶ Process mitigation steps enabled through sensor redundancy available for both pH and DO sensor technology



Control Software

- ▶ Validated control software, can be connected via OPC UA server to SCADA (supervisory control and data acquisition) system
- ▶ Developed in accordance with GAMP5
- ▶ Easy and safe operation in stand-alone mode or integrated into supervisory system



Applications

Typical applications include:

- ▶ Mammalian suspension cell culture, using e.g. CHO or HEK293 cells
- ▶ Insect cell culture, using e.g. Sf9 cells
- ▶ Suspension cells in batch and fed-batch
- ▶ Adherent cell growth on microcarriers, ex. VERO

Engineering Characterization

Pall has conducted a range of calculations and experiments to characterize the Allegro STR bioreactors. Mixing characterization testing methods, such as mixing time, fluid velocity profiles and Kolmogorov scale were assessed in Computational Fluid Dynamics studies (CFD). The mixing model was then verified in a tracer experiment. Other important parameters like $k_{L,a}$, dissolved CO₂ removal and heating/cooling profiles with thermocouples inserted were also performed. The experimental setup and results are documented in a separate application note.

Geometry

When filled with the maximum working volume, the Allegro STR bioreactors have an aspect ratio of approximately (1.4:1). This significantly reduces the overall height of the bioreactor compared to cylindrical versions of the same working volume. It is also much easier and safer for the operators to perform most installation steps without the use of ladders, hoists, or platforms.

The filled biocontainer in the bioreactor forms in a cubical design. The cubical design facilitates mixing as natural baffles and increase the turbulence in the bioreactor. Additional baffles have been placed at three sides of the container to further increase that effect, especially at the high end of agitation speed. Pall has performed extensive studies using CFD to show that there are no dead zones and 95% homogenization can be reached within less than 16 seconds (full impeller speed at up to 2000 L working volume).



Bioreactor Hardware

The bioreactor hardware provides the support for the biocontainer. It has been designed with a viewing window to observe the cell culture at any time. Heating is provided via a water jacket on all Allegro STR bioreactors. On the Allegro STR 200 bioreactor, an option with a heating mat is also available. Pall offers a Temperature Control Unit (TCU) to work with the Allegro STR bioreactors and the integration of other common TCUs can be discussed. The Allegro STR bioreactor portfolio also can be equipped to communicate with third party TCUs. The bioreactor hardware has integrated load cells to provide information on the liquid mass.



Controller

The controller uses a Siemens TIA Portal platform with a color touch screen Panel PC (SIMATIC IPC 477D) HMI. The controller can connect to higher networks via OPC.

Operator interaction with the bioreactor is via a large HMI color screen. Industrial design guidelines were used to provide all the information in an intuitive and well-arranged format on screen.



Gas Flow Control

All biocontainers have the option to provide gas through the sparger, into the head space overlay and into a separate open pipe sparger for additional CO₂ removal. The controller hosts 6 digital thermal mass flow controllers to control the gas flow. Four of them are calibrated to assure the proper mix of air, oxygen, nitrogen and carbon dioxide through the main sparger. Another is calibrated for air on the CO₂ removal line. For the overlay gas, the flow rate of the 4-gas mix is also recorded by a digital mass flow meter and a bank of solenoid valves. The gas mix is in this case created by pulsing up to 4 different gasses (air, oxygen, nitrogen and carbon dioxide) into the biocontainer.

Liquid Dosing

The tubing management system, when fully inflated, holds all bioreactor addition lines with sterile connectors directly over the pump heads of the hardware. The Allegro STR bioreactors are equipped with four integrated pumps for liquid dosing. These cover the flow ranges that are needed for the respective cascades (base, glucose, antifoam, etc.). For the addition of larger liquid volumes, e.g. during filling or inoculation, external pumps can be controlled via analog signals. All pumps, integrated and external, can be made part of a defined control cascade.



Sensor Configurations

- ▶ Liquid temperature within the biocontainer is measured using a Pt100 temperature sensor, this is surface mounted on the Allegro STR 50 bioreactor
- ▶ Internal pressure within the biocontainer is measured using a single-use pressure sensor supplied with each single-use system
- ▶ Level sensors in the hardware control the door interlocks and temperature control to ensure there is a minimum liquid volume prior to heating starting
- ▶ Transmitters for conventional pH and DO sensors are integrated in the controller and redundant transmitters are available on selected models

- ▶ Spare analog inputs (4-20 mA signal) can be used to connect external pumps, balances and sensors without modifications on the hardware. The ancillary inputs added also are configured on the HMI
- ▶ Single-use sensors for pH and DO are available on selected models



Electronic Records (21 CFR 11)

The Allegro STR bioreactor controller offers all the technical features that are required to run the system compatible with 21 CFR 11. An administrator can set up users and define user rights and password. The password safety complies with the requirement in 21 CFR 11 for electronic signatures. The electronic records are saved within the system and options for backup to external systems are available. The controller also offers an OPC interface to fully control the operations on the bioreactor from an external SCADA system.

Single-Use Biocontainer

The biocontainer is made of the transparent, low extractable Allegro film as all other Allegro single-use systems, and which has been well characterized for extractables and leachables. The biocontainers are 100% integrity tested during manufacture. All ports and connections are labeled with colored number tags, providing guidance on how to set up and connect the biocontainer to the hardware. Each system is provided with multiple liquid addition lines and all are fitted with Kleenpak® sterile connectors or Kleenpak Presto genderless sterile connectors as standard.



Allegro STR 1000 biocontainer



Allegro STR 50 biocontainer with Kleenpak Presto sterile connectors shown on the single-use biocontainer

Biocontainer Packaging

In many manufacturers' current use of a single-use bioreactor design, there is risk of damage to the bioreactor bag during transportation and installation. It is therefore very important that the biocontainer is properly packaged and the interaction of the operator with the equipment is well defined. Protection of the single-use bioreactor and the process it was being used in were considered from the outset of the Allegro STR bioreactor development.

For each size of the Allegro STR biocontainers, Pall has designed innovative specific protective packaging. Some components of the packaging are integral to the single-use system and provide support for the connectors, tubing and filters in the single-use system, thus reducing the risk of damage to the film during transport. On the larger systems, the packaging material provides ergonomic handles and guides to easily move and position the single-use systems during unpacking and installation.



Impeller and Sparger Assembly

The Allegro STR bioreactors provide mixing via a bottom mounted pitched blade 'elephant ear' impeller. This shape has been selected from many candidates for good performance in mammalian cell culture. The three 45 degree angle blades promotes efficient axial and radial mixing, while supporting options for both upward and downward flow depending on the application required.

The impeller is directly connected to the motor, which allows excellent power transfer of approximately 0.25 W/kg into the liquid. A dynamic seal arrangement at the bottom of the biocontainer assures leak-free operation up to the claimed service life.

The ring sparger and the CO₂ removal line are mounted directly under the impeller. This supports the effective distribution of the gas bubbles in the liquid and increases their residence time in the bioreactor. The sparger lines are fitted with sterilizing grade Pall Emflon II gas filters. This design facilitates further effective gas bubble distribution from the gas to liquid interface, maximizing mass transfer required to support cell culture requirements.

Customization

Pall has the standard biocontainer equipped with all liquid and gas connections for the most typical processes. Most operations, can be set up with the standard biocontainer. All ports are fitted with sterile connections. Process liquids, including media and inoculation, can easily and safely be transferred into the bioreactor.

Pall can assist in customizing the bioreactor systems. This includes small changes on the biocontainer up to integrating the bioreactor with all surrounding unit operations into a fully automated process.



Setting up a transfer from an Allegro STR 50 L bioreactor to an Allegro STR 200 L bioreactor

Documentation and Certificates

The Allegro STR bioreactors are 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, where applicable.

Pall also can assist with hardware qualification, and standard Installation Qualification (IQ), Operational Qualification (OQ), FAT, and Site Acceptance Test (SAT) test protocols are available.

Pall Quality Standards

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

- ▶ Good Automated Manufacturing Practice (GAMP) current version
- ▶ CFR21 Part 11 for electronic records
- ▶ Electromagnetic Compatibility (EMC) to EN 61326-1: 2013 and FCC CFR 47 Part 15B: 2013
- ▶ CE Marking to European Directives 2014/30/EU (2004/108/EC extant until April 2016) and 2014/35/EU (2006/95/EC extant until April 2016)
- ▶ 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, please contact your local Pall cell culture specialist.

The Allegro STR single-use biocontainers quality standards include:

- ▶ The product is made in a manufacturing facility where the Quality Management System is ISO13485 & ISO9001 certified
- ▶ 100% of Allegro STR 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 STR biocontainers and seal housings undergo a pressure leak test during manufacturing
- ▶ Samples from each biocontainer manufacturing batch undergo a tensile test of the film weld area & 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> Bacterial Endotoxins Test 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
- ▶ The materials of construction of the Allegro STR biocontainer meet:
 - USP<88> Biological Reactivity Test *In Vivo* for Class VI - 50 °C Plastics
 - USP<87> Biological Reactivity Tests *In Vitro*, Cytotoxicity
 - USP<85> Bacterial Endotoxin Tests
 - USP<661> Physico-chemical Testing for Plastics
 - USP<788> Particulate Matter in Injections

- ▶ Materials of construction of all components in contact with fluid are certified TSE/BSE risk materials free
- ▶ Are supplied double bagged and irradiated at a minimum dose of 25 KGy



Preventive Maintenance and Service Packages

The Allegro STR single-use bioreactors 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's AcceleratorSM 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 STR bioreactors, minimizing downtime. These include a full functional test, exchange of wear parts and calibration of all sensors.



Technical Data

	Allegro STR 50 Bioreactor	Allegro STR 200 Bioreactor	Allegro STR 500 Bioreactor	Allegro STR 1000 Bioreactor	Allegro STR 2000 Bioreactor
Part number	STR50-JC230 ¹	STR200-JC230 ²	STR500-JC (110/230)	STR1000-JC	STR2000-JC
Operational system space requirements (W x D x H)	1330 x 950 x 1500 mm	1620 x 1162 x 2120 mm	1340 x 1300 x 2345 mm	1530 x 1493 x 2554 mm	1780 x 1740 x 2930 mm
Weight (empty)	240 kg	720 kg	721 kg	930 kg	1320 kg
Working volume	10 – 50 L	60 – 200 L	100 – 500 L	300 – 1000 L	400 – 2000 L
Impeller speed	25 to 200 rpm	25 to 150 rpm	25 to 150 rpm	25 to 105 rpm	25 to 105 rpm
Temperature range	4 to 40 °C	4 to 40 °C	4 to 40 °C	4 to 40 °C	4 to 40 °C
pH control	2 – 12 pH	2 – 12 pH	2 – 12 pH	2 – 12 pH	2 – 12 pH
DO control	0 – 200% air saturation	0 – 200% air saturation	0 – 200% air saturation	0 – 200% air saturation	0 – 200% air saturation
Conventional probe ports	6	4	6	6	6
Conventional probe transmitters	2 x pH, 2 x DO	2 x pH, 2 x DO	2 x pH, 2 x DO	2 x pH, 2 x DO	2 x pH, 2 x DO
Single-use sensor ports	2 x pH, 2 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO
Single-use sensor transmitters	2 x pH, 2 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO	1 x pH, 1 x DO
Temperature sensor	1 x Pt100	1 x Pt100	1 x Pt100	1 x Pt100	1 x Pt100
Analogue inputs	4	2	4	4	4
Load cell range	0 – 60 kg	0 – 250 kg	0 – 550 kg	0 – 1100 kg	0 – 2500 kg
Connections for external balances	4	2	2	2	4
Internal pumps	4	4	4	4	4
Connections for external pumps	2	1	2	2	2
Pump speed	0 – 408 rpm	0 – 100 rpm	2 x 0 – 100 rpm 2 x 0 – 480 rpm	2 x 0 – 100 rpm 2 x 0 – 480 rpm	2 x 0 – 100 rpm 2 x 0 – 480 rpm

NOTE:

¹Available with 110 V version (STR50-JC110); Allegro STR 50 bioreactor system available with standard second set single-use sensor transmitters (STR50-JC230-R-SU & STR50-JC110-R-SU)

²Allegro STR bioreactor system also available with 110 V version (Part number STR200-JC110)

Ordering Information

The Allegro STR bioreactors are available as standard models and both the bioreactor hardware and consumables can be customized. Please contact a Pall representative to find the ideal solution for your application. 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

Please contact Pall for a Total Solution discussion on your process.





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