PALL CORPORATION

Lentivirus (LV) Adherent Platform Accelerator℠ Integrated Solutions

USD 3609

Contents

Illustrations and recommendations are based upon a typical process and may be subject to change based upon your individual process requirements.

LV Drug Products

An introduction to today's LV manufacture

Encouraging results from gene-modified cell therapies such as CAR-T cell-based therapy, have been reported, and there is growing optimism that similar therapies will have a big impact on patient health. In the case of CAR-T cell therapy, the T-cells are modified with a viral vector. Commonly these are Lentivirus (LV), and these offer unique advantages over other gene delivery systems, namely the ability to integrate transgenes into the genomes of both dividing and nondividing cells. The large packaging capability of the LV allows the *in-vivo* or *in-vitro* transfer of large encoding sequences (genes) that are not possible with other commonly used vectors such as adeno-associated virus (AAV). Gene therapies represent a new medical paradigm and their astonishing potential is the result of decades of research, acquired best practices, and hard lessons learned. Despite an increasing number of gene therapy drugs in development and clinical trials every year, this industry is still in its early stages. While there is a lot of experience from the field of recombinant antibodies that can be bridged to this evolving industry, manufacturers are facing differences and challenges at various stages of the drug marketing journey, from development and manufacture, to the regulatory approval of LV for *in-vitro* and *in-vivo* use.

Plan for Speed in LV Development

In process development, speed-to-market and cost are critical considerations. Selecting the most suitable production system that results in fast production, high yields, and high potency of LV is key. Several producers are currently entering the market using transfection and adherent cells because it presents a fast route to market. There are, however, limitations to adherent based manufacture, as typical LV titers currently plateau around 10^6-10^7 transduction units (TU) per cm^2. For products that require higher yield and lower costs, adapting cells for suspension culture or developing stable producer lines comes with a long-term benefit regarding yield and scalability.

Increase Capacity and Quality in LV Manufacture

The global viral vector manufacturing capacity is estimated to be 1–2 orders of magnitude lower than what is needed to support commercial supply requirements both today and in future. Academics and industry are therefore putting focus on measures for the most durable capacity increase; improving manufacturing practices to increase productivity through engineering cell lines, refining plasmid constructs, and enhancing process recovery in downstream processing. Current downstream recovery yields are at only 15%, and maximizing yield while meeting both product and impurity specifications is a significant challenge in downstream. Lentivirus is extremely sensitive, and this makes the downstream processing a race against the clock. Although yields at bench scale can be higher, techniques such as ultracentrifugation are not scalable. Other techniques need therefore to be used such as filtration, chromatography, and tangential flow filtration. This forces manufacturers to work extremely quickly, and to find ways to protect the product as much as they can.

Gain Regulatory Approval for LV Drug Products

Moving LV products into the highly regulated GMP production environment puts a spotlight on the rising expectations in drug reviews, as the understanding in industry and regulatory bodies is building. The much-needed regulatory framework is rapidly developing and is supported through multiple guidance documents that have been published recently. Part of the challenge to obtain regulatory approval is paired with analytical constraints: viral titer, quality and impurity assays require lengthy off-line processes and come with limited sensitivity. This burden is lighter when LV is used as raw material for gene modified cell therapies, but remains important for applications with *in-vivo* use. A series of next generation analytical tools are being developed at rapid pace and promise real-time monitoring and implementation of process analytical technology (PAT).

With several approved gene therapies and hundreds of products in the pipeline, the industry knowledge is building quicky. At the same time, the regulatory framework is rapidly developing which is increasing confidence in interpreting guidelines and builds maturity in both industry and within those regulatory bodies. The continued connection of academic research, industry investment, and regulatory commitment comes with great potential for streamlining LV manufacturing and allows new, high-quality LV therapeutics and gene modified cell therapies to reach patients.

1. Cell Seeding

Additional resources

Early in therapeutic development, viral vectors are routinely manufactured in adherent cells using two-dimensional systems such as cell factories or roller bottles. As processes scale-up and more virus is needed, the footprint and manual operations required to grow cells in this fashion quickly becomes problematic and formats more suited to the industrialization of the process become advantageous.

Equipment

Xpansion® Multiplate Bioreactor System

The Xpansion multiplate bioreactor system is a closed, single-use system that has been specifically designed for the scale-up of shear-sensitive, adherent mammalian cells under controlled conditions. Its 2D multiplate design allows easy scale-up from traditional multitray systems.

Part Number (PN): XPNBRS

Discover more

Input

Allegro™ Bioprocessing Workstations
PN: LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC

Transfer Sets
PN: 7292-1398X, 7292-1397E

Xpansion Seeding Manifolds
PN: 7414-0972X

Allegro® 2D Standard Systems
PN: 7190-1397S

Nunc• Cell Factory• System

T-Flasks

Output

Allegro 2D Standard Systems
PN: 7190-1397U

Supporting

mPath™ Benchtop Bioreactor Control Tower
PN: MPATHLINK

Xpansion Lift
PN: XPNLIFT

Xpansion 100 Bioreactor
PN: XPN50

Xpansion Harvest Station
PN: XPNHVST

When viewing on desktop roll over product name to view more

2. Cell Culture

Additional resources

As the therapies progress into clinical development, there is a requirement to increase the quantity of the virus produced while maintaining the quality. Manufacturing virus from adherent cells using fixed-bed bioreactor technology allows for direct transfer of the 2D reference process, simplifying technology transfer, minimizing risk and saving time.

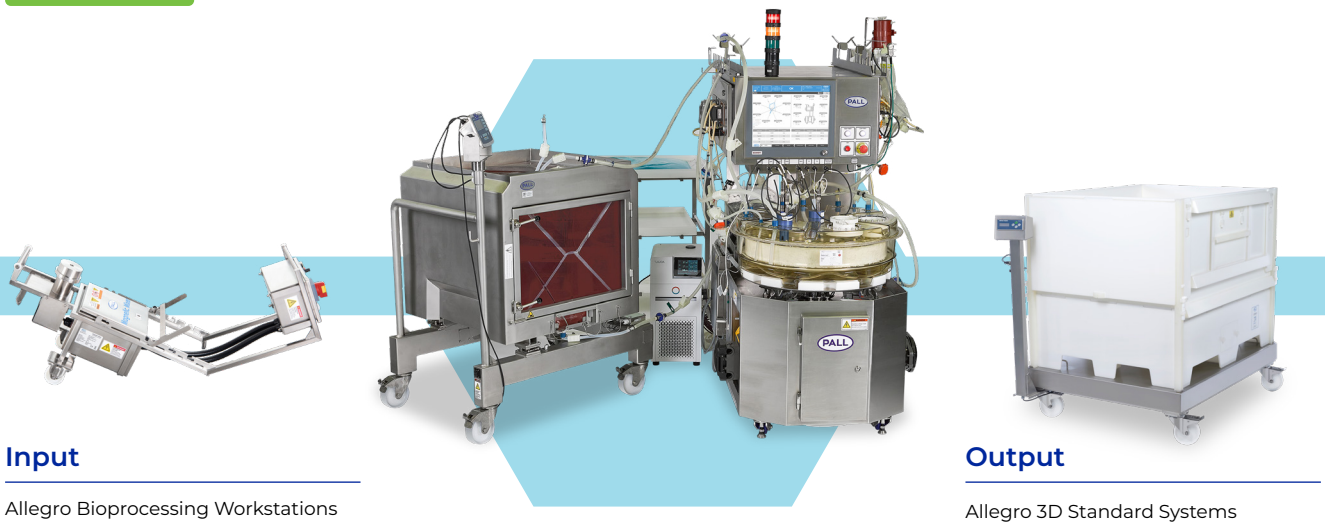
Equipment

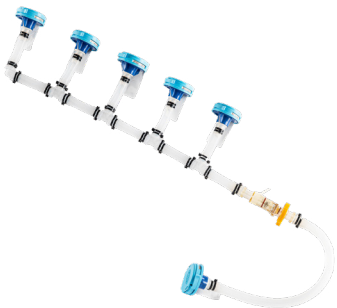
iCELLis® 500+ Bioreactor

The iCELLis bioreactor was the world's first fully integrated high-cell density bioreactor designed to simplify processes by combining the advantages of both single-use technology and a fixed-bed system to simplify cell culture and reduce operational costs. The system delivers a scalable platform to support cell culture from R&D to industrial scale GMP manufacturing.

PN: ICL500CSSSIPH

Discover more





Input

Allegro Bioprocessing Workstations
PN: LGRTBDC, LGRTSDC, 215-19658-B4N, LGRTPE20L, LGRTLPE20L, LGRTRDC

Allegro 3D Standard Systems
PN: 7190-1374Y

Allegro Plastic Totes
PN: LGRPTTE200L, LGRPTRL200L, LGRPTTEL200L, LGRUFBK

Emflon® II Membrane in Kleenpak™ Capsules
PN: 7090-1388M

LevMixer® System
PN: LM200JCMA-B4N, LMG403, 7403-1352K

Magnetic Mixer
PN: MMG403, 7404-1401R, LM200JCMA-B4N

Transfer Sets
PN: 7292-1381X, 7292-1381Q

Output

Allegro 3D Standard Systems
PN: 7190-1376T, 7190-1374Y

Allegro Plastic Totes
PN: LGRPTTE200L, LGRPTRL200L, LGRPTTEL200L, LGRPTTE500L, LGRPTTEL500L, LGRPTRL500L

LevMixer System
PN: LM200JCMA-B4N, LMG403, 7403-1352K

Transfer Sets
PN: 7292-1381X

Supporting

Allegro 2D Standard Systems
PN: 6415-0615S, 6415-0615T, 7190-1397P, 7190-1376R

iCELLis 500+ Bioreactor Manifolds
PN: 6415-0464G, 6415-0615U

iCELLis 500+ Bioreactor Vessel
PN: 4415-I500H133

Transfer Sets
PN: 7292-1382M, 7292-1381U, 7292-1381X

When viewing on desktop roll over product name to view more

3. Clarification

Additional resources

The clarification step removes cells, cell debris, and other impurities to reduce biological burden. The easiest and most economical technology to clarify the cell culture is filtration. As cell densities increase upstream, maintaining high yields becomes more of a challenge. Our experienced technical team can help you find the best filter technologies and protocols for your process, whether you are just beginning process development or need to re-evaluate and optimize this step down the line. Supor® membranes are appropriate for this step and are available in a wide range of sizes and formats. Contact us for assistance in determining the best filter technology for your process.

Equipment

Allegro MVP Single-Use System

A fully automated bioprocessing system, providing flexibility and improved productivity in upstream and downstream single-use processing.

PN: LGRMVPAPE, CBG401A

Discover more

Input

LevMixer System
PN: LMG403, LM200JCMA-B4N, 7403-1352K

Allegro Bioprocessing Workstations
PN: LGRKPCBKHD, LGRUFBK, LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC

Allegro 2D Standard Systems
PN: 7190-1397M

Transfer Sets
PN: 7292-1381X

Supporting

Supor EAV Membrane in Kleenpak Capsules
PN: 7090-1562Q

Transfer Sets
PN: 9430-1413G, 9430-1413Q, 7292-1381A

Output

Transfer Sets
PN: 7292-1381X

LevMixer System
PN: 7403-1352N, LM400JCMA-B4N, LMG403

Allegro 2D Standard Systems
PN: 7190-1397N, 7190-1397S

Allegro Bioprocessing Workstations
PN: LGRTPE20L, LGRTLPE20L, LGRTRDC

When viewing on desktop roll over product name to view more

4. Purification (Chromatography)

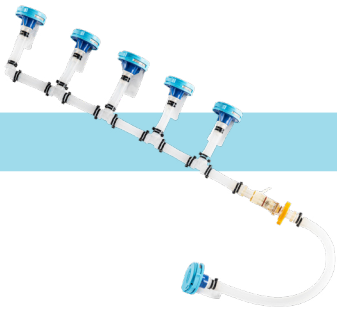
Additional resources

Purification by ion exchange chromatography can be optimized to reduce empty capsids, DNA and host cell proteins (HCP). Adsorptive membrane technology offers an efficient and effective option for this step and, as a scalable single-use solution, is quickly integrated into any manufacturing process.

Equipment

ÄKTA ready† Single-Use System

ÄKTA ready is a single-use liquid chromatography system built for process scale-up and manufacturing. The system uses disposable flow paths and pre-packed columns that enable flexibility and speed in bioprocessing.



Input

LevMixer System
PN: LMG403, 7403-1352N, LM400JCMA-B4N

Allegro Bioprocessing Workstations
PN: LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC

Allegro 2D Standard Systems
PN: 7190-1397P, 7190-1397M, 7190-1397N

Transfer Sets
PN: 7292-1381L

Output

Allegro Plastic Totes
PN: LGRPTTE500L, LGRPTRL500L, LGRPTTEL500L

Transfer Sets
PN: 7292-1381L

Allegro 2D Standard Systems
PN: 7190-1397S

Allegro 3D Standard Systems
PN: 7190-1376T

Supporting

Transfer Sets
PN: 7291-1399Y

UNICORN† Workstation License

ÄKTA ready Low Flow Kit

Mustang® Q XT Ion Exchange Chromatography Capsules
PN: XT5000MSTGQP1V, XT5000B100, XT5000H100, XT5000T100

When viewing on desktop roll over product name to view more

5. Concentration

Additional resources

Ultrafiltration (UF)/diafiltration (DF) using tangential flow filtration (TFF) membranes further concentrates the target molecule and exchanges buffers. Achieving high yield is a common challenge with TFF, but our technical experts can work with you to optimize your UF/DF unit operation and system design based on years of experience and thorough testing.

Equipment

ÄKTA readyflux† Filtration System

The automated system uses gamma-irradiated single-use flow kits including single-use flow path, pumps and sensors for pressure, conductivity, temperature, flow and pH. These allow a wide variety of control modes and help users to tailor the filtration controls to different processing requirements.

Input

Allegro Bioprocessing Workstations
PN: LGRTBDC, LGRTRDC, LGRTSDC, LGRTPE20L, LGRTLPE20L

Allegro 2D Standard Systems
PN: 7190-1397S, 7190-1397M, 7190-1397N

Transfer Sets
PN: 7292-1381A

Output

Allegro 2D Standard Systems
PN: 7190-1397N, 7190-1397S

Allegro Bioprocessing Workstations
PN: LGRTPE20L, LGRTLPE20L

Transfer Sets
PN: 7292-1381A

Supporting

Allegro 2D Standard Systems
PN: 7190-1397N

Cadence® Single-Use Tangential Flow Filtration (TFF) Modules
PN: CSUM100T010, 7443-1437P

Transfer Sets
PN: 7292-1381A

ÄKTA readyflux Flow Kit TriClamp

Bagkart† Bag Trolley

C10 Manifold with TriClamp ready for readyflux

UNICORN† Workstation License

When viewing on desktop roll over product name to view more

6. Bulk Filtration

Additional resources

Manufacturing clinical material requires the production of highly pure and biologically active vectors that meet regulatory requirements. As part of this, a final sterilizing grade (0.2 µm) filtration step contributes to sterility assurance and maintains product safety while the product is sent for final filling.

Equipment

Palltronic® Flowstar V Filter Integrity Test Instrument

The Palltronic Flowstar V integrity test instrument ensures accurate filter integrity testing with a further reduction in test time, full compliance with 21 CFR Part 11, advanced automation capabilities, and simplified network integration saving the user time while improving process efficiency.

PN: FFS05

Discover more

Input

Allegro 2D Standard Systems
PN: 7190-1397N, 7190-1397M

Transfer Sets
PN: 7292-1382M

Supporting

Supor EKV Sterilizing-Grade Membrane in Mini Kleenpak Capsules
PN: 7090-1388G

Emflon II Membrane in Mini Kleenpak Capsules

When viewing on desktop roll over product name to view more

Additional Resources

Process Description	Format	Title	Link
LV Adherent	Poster	Scaling-Up and Industrializing the Production of Viral Vectors and Cells for Therapeutic Use	
LV Adherent	Poster	Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector	
Cell Seeding	Poster	Scaling-Up and Industrializing the Production of Viral Vectors and Cells for Therapeutic Use	
Cell Seeding	Poster	Overcoming Adherent Seed Train Biomass Limitations: Pall Xpansion Bioreactor	
Cell Culture	Video	iCELLis Single-Use Fixed-Bed Bioreactors	
Cell Culture	Poster	Adherent HEK293t Cells Cultured in Pall's iCELLis Bioreactor with OptiPEAK HEK293t Blood-Free Chemically Defined Media Exhibit Robust and Rapid Population Doubling Times	
Clarification	Poster	Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector	
Clarification	Poster	Optimizing the Clarification of Viral Vector Culture for Gene Therapy	
Purification (Chromatography)	Poster	Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector	
Purification (Chromatography)	White Paper	Mustang Membrane Chromatography for Gene Therapy Purification: A Robust and Scalable Solution	
Concentration	White Paper	Understanding Single-Pass Tangential Flow Filtration and the New Era of Bioprocessing	
Concentration	Poster	Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector	
Bulk Filtration	Poster	Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector	

Equipment List

Step No.	Process Description	Product	Part Number	Link
1	**Cell Seeding**	Nunc Cell Factory System	**N/A**	
1	**Cell Seeding**	T-Flasks	**N/A**	
1	**Cell Seeding**	Allegro Bioprocessing Workstations	**LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC**	▶
1	**Cell Seeding**	Transfer Sets	**7292-1398X, 7292-1397E**	
1	**Cell Seeding**	Xpansion Seeding Manifolds	**7414-0972X**	▶
1	**Cell Seeding**	Allegro 2D Standard Systems	**7190-1397S, 7190-1397U**	▶
1	**Cell Seeding**	Xpansion Multiplate Bioreactor System	**XPNBRS**	▶
1	**Cell Seeding**	mPath Benchtop Bioreactor Control Tower	**MPATHLINK**	▶
1	**Cell Seeding**	Xpansion Lift	**XPNLIFT**	▶
1	**Cell Seeding**	Xpansion 100 Bioreactor	**XPN50**	▶
1	**Cell Seeding**	Xpansion Harvest Station	**XPNHVST**	▶
2	**Cell Culture**	Allegro 2D Standard Systems	**6415-0615S, 6415-0615T, 7190-1397P, 7190-1376R**	▶
2	**Cell Culture**	Allegro 3D Standard Systems	**7190-1376T, 7190-1374Y**	▶
2	**Cell Culture**	Allegro Bioprocessing Workstations	**LGRTBDC, LGRTSDC, 215-19658-B4N, LGRTPE20L, LGRTLPE20L, LGRTRDC**	▶
2	**Cell Culture**	Allegro Plastic Totes	**LGRPTTE200L, LGRPTRL200L, LGRPTTEL200L, LGRPTTE500L, LGRPTTEL500L, LGRPTRL500L, LGRUFBK**	▶
2	**Cell Culture**	Emflon II Membrane in Kleenpak Capsules	**7090-1388M**	▶
2	**Cell Culture**	iCELLis 500+ Bioreactor	**ICL500CSSSIPH**	▶
2	**Cell Culture**	iCELLis 500+ Bioreactor Manifolds	**6415-0464G, 6415-0615U**	▶
2	**Cell Culture**	iCELLis 500+ Bioreactor Vessel	**4415-I500H133**	▶
2	**Cell Culture**	LevMixer System	**LM200JCMA-B4N (EU), LM200JCMA-B4A (US), LMG403, 7403-1352K**	▶
2	**Cell Culture**	Magnetic Mixer	**MMG403, 7404-1401R, LM200JCMA-B4N (EU), LM200JCMA-B4A (US)**	▶
2	**Cell Culture**	Transfer Sets	**7292-1381X, 7292-1381Q, 7292-1382M, 7292-1381U, 7292-1381X**	

Equipment List

Step No.	Process Description	Product	Part Number	Link
3	**Clarification**	LevMixer System	**LMG403, LM200JCMA-B4N (EU), LM200JCMA-B4A (US), 7403-1352K, 7403-1352N, LM400JCMA-B4N (EU), LM400JCMA-B4A (US), LMG403**	
3	**Clarification**	Allegro Bioprocessing Workstations	**LGRKPCBKHD, LGRUFBK, LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC**	
3	**Clarification**	Allegro 2D Standard Systems	**7190-1397M, 7190-1397N, 7190-1397S**	
3	**Clarification**	Transfer Sets	**7292-1381X, 9430-1413G, 9430-1413Q, 7292-1381A**	
3	**Clarification**	Allegro MVP Single-Use System	**LGRMVPAPE, LGRMVPAPA, CBG401A**	
3	**Clarification**	Supor EAV Membrane in Kleenpak Capsules	**7090-1562Q**	
4	**Purification (Chromatography)**	LevMixer System	**LMG403, 7403-1352N, LM400JCMA-B4N (EU), LM400JCMA-B4A (US)**	
4	**Purification (Chromatography)**	Allegro Bioprocessing Workstations	**LGRTBDC, LGRTSDC, LGRTPE20L, LGRTLPE20L, LGRTRDC**	
4	**Purification (Chromatography)**	Allegro 2D Standard Systems	**7190-1397P, 7190-1397M, 7190-1397N, 7190-1397S**	
4	**Purification (Chromatography)**	Transfer Sets	**7292-1381L, 7291-1399Y**	
4	**Purification (Chromatography)**	ÄKTA ready Single-Use System	**29032038**	
4	**Purification (Chromatography)**	Allegro Plastic Totes	**LGRPTTE500L, LGRPTRL500L, LGRPTTEL500L**	
4	**Purification (Chromatography)**	UNICORN Workstation License	**29128116**	
4	**Purification (Chromatography)**	ÄKTA ready Low Flow Kit	**28930182**	
4	**Purification (Chromatography)**	Mustang Q XT Ion Exchange Chromatography Capsules	**XT5000MSTGQP1V, XT5000B100, XT5000H100, XT5000T100**	
4	**Purification (Chromatography)**	Allegro 3D Standard Systems	**7190-1376T**	

Equipment List

Step No.	Process Description	Product	Part Number	Link
5	**Concentration**	ÄKTA readyflux Filtration System	**29151000**	
5	**Concentration**	ÄKTA readyflux Flow Kit TriClamp	**29151600**	
5	**Concentration**	Allegro 2D Standard Systems	**7190-1397S, 7190-1397M, 7190-1397N**	▶
5	**Concentration**	Allegro Bioprocessing Workstations	**LGRTBDC, LGRTRDC, LGRTSDC, LGRTPE20L, LGRTLPE20L**	▶
5	**Concentration**	Bagkart Bag Trolley	**29151500**	
5	**Concentration**	C10 Manifold with TriClamp ready for readyflux XL	**7443-1437S**	
5	**Concentration**	Cadence Single-Use Tangential Flow Filtration (TFF) Modules	**CSUM100T010, 7443-1437P**	▶
5	**Concentration**	Transfer Sets	**7292-1381A**	
5	**Concentration**	UNICORN Workstation license	**29128116**	
6	**Bulk Filtration**	Supor EKV Sterilizing-Grade Membrane in Mini Kleenpak Capsules	**7090-1388G**	
6	**Bulk Filtration**	Allegro 2D Standard Systems	**7190-1397N, 7190-1397M**	▶
6	**Bulk Filtration**	Transfer Sets	**7292-1382M**	
6	**Bulk Filtration**	Palltronic Flowstar V Filter Integrity Test Instrument	**FFS05**	▶
6	**Bulk Filtration**	Emflon II Membrane in Mini Kleenpak Capsules		▶

Scientific and Laboratory Services

The scientific and regulatory knowledge that supports the selection, adoption and ongoing use of critical process technology, coupled with analytical, imaging and measurement capabilities, creates a versatile and practical resource ready to respond to an ever-changing industry. Pall duplicates these laboratories across the globe and leverages their cumulative knowledge to deliver practical scientific and regulatory support to all process technologies to keep you moving forward.

Technical Services

The accessibility of local technical support networks minimize delays in your journey at all points. From the early stage of process development to on-site support for mature processes, Pall's technical support groups are there to help remove barriers to progress and to make your journey as rapid and stress-free as possible. Our knowledge of the technology and the process can be applied to everything from training to troubleshooting and consultancy. Our global team of technology experts are on hand to respond to your changing needs.

Advanced Separation Systems

Operating within the defined design space demands the monitoring and control of critical process parameters to assure product quality. Systems that control critical unit operations and that communicate with your existing process components can control process risks and maximize productivity by reducing operator involvement for many processes, Pall applies strong engineering and regulatory understanding to deliver compliant and qualified systems that safeguard and simplify your journey.

Process Development Services

Prior knowledge is a rare and valuable commodity, especially when preparing to take a new direction or when under pressure to deliver to a tight deadline. Take advantage of Pall's experience, process knowledge and technical know-how to help you achieve your goals. From the optimization of an end-to-end continuous process to establishing the right parameters for a single unit operation, our teams of scientists are ready to work with you and to generate the data you need to make the critical decisions necessary for success.

Validation Services

Arriving at your destination counts for nothing without the necessary paperwork to proceed to the next stage. Pall's Validation Services are committed to delivering the supporting data packages and analysis required to quantify process risk and to support regulatory submission. Our strengths include critical filtration technologies such as the performance validation of sterilizing grade filtration, and we are at the forefront of the evolving needs in the area of extractables and leachables for all product contact components. We combine the generation of data with interpretation and consultancy to deliver data packages that are ready for regulatory scrutiny and to ensure there are no barriers to progress.

Servicing and Maintenance

Our range of service packages keeps your equipment protected and well maintained, and includes itemized pay-as-you-go services, start-up care and training packages and a variety of post-warranty service plans that include priority response times, discounts for emergency repairs and flexible payment options. Pall service plans provide total peace of mind and worry-free support throughout the coverage period.

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