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The Oligonucleotide Purification & Concentration Checklist

Addressing Next-Generation Technologies for Refining Oligos in Your Facility

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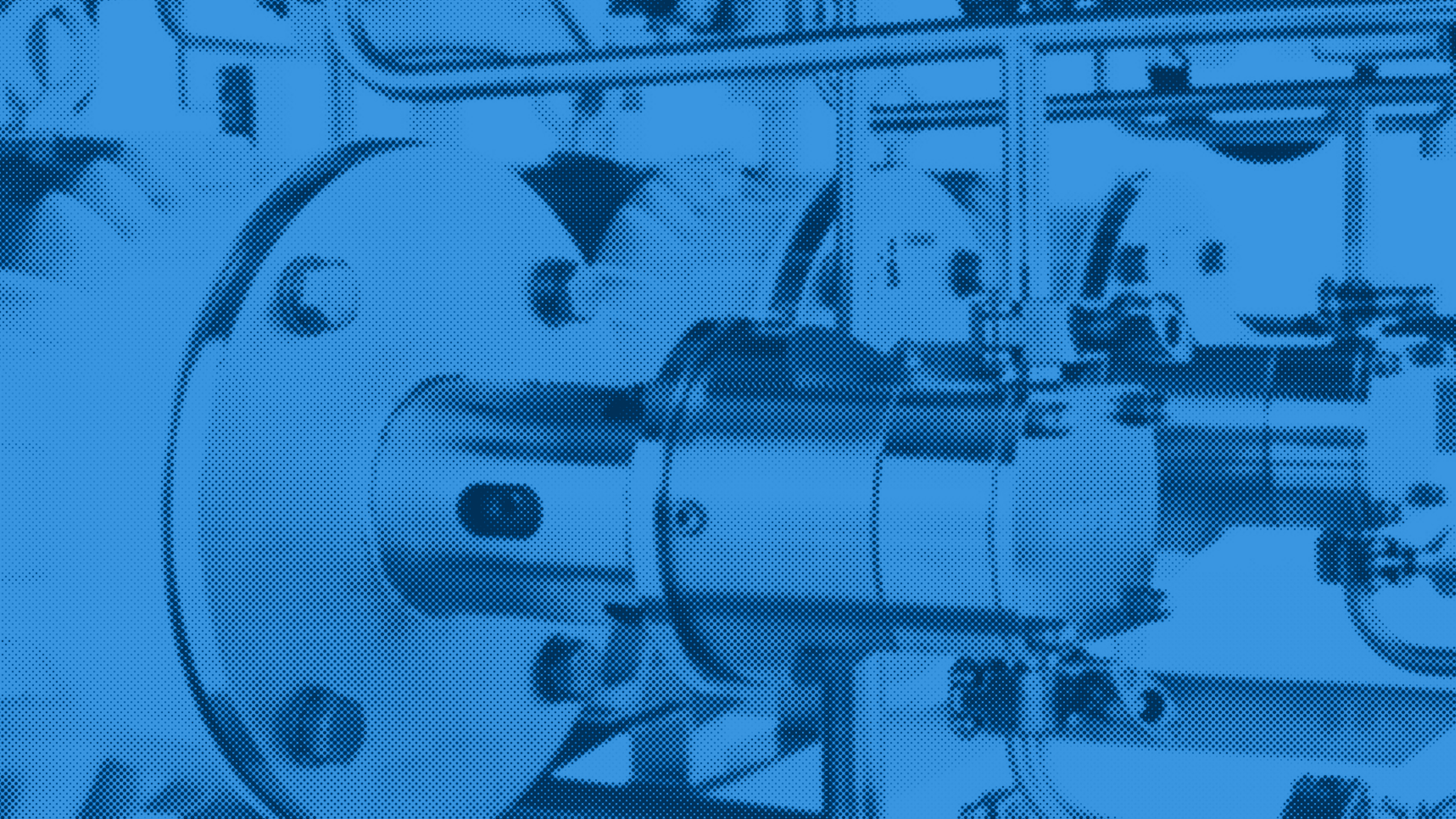


Introduction

Oligonucleotides, or oligos, are in high demand because of their use in research, therapeutics, drug development and other applications. As a result, biomanufacturers are increasingly looking for new purification and concentration technologies that help increase the yield and quality of their oligos. In this checklist, our oligo experts offer guidance on what factors to consider when upgrading your equipment and components.

Oligos are short sequences of nucleotides that are the building blocks of DNA and RNA. They can be custom-designed for specific applications, making them versatile tools in scientific research, medical diagnostics, and therapeutic developments.

Oligo demand has grown in recent years and is expected to reach a value of $17 billion by 2032. Biomanufacturers are taking notice and looking for better ways to increase oligo throughput. One way is to improve their purification and concentration techniques.

Purification removes impurities and undesired components to obtain a highly pure and specific oligo product. Efficient and optimized purification steps are essential because they directly impact the amount of usable oligo obtained for downstream applications.

More efficient chromatography enhances the yield of oligo development by removing impurities and undesired byproducts from the synthesis process.

Concentration increases the amount of oligo per unit volume or weight and is achieved through filtration. It plays a significant role in the economic aspect of production, as it allows for more product units per measure and reduces costs associated with storage, shipping, and administration. This is particularly important in applications where space is limited.

Traditionally, biomanufacturers have focused on the speed of their oligosynthesis process because it is time sensitive. However, the subsequent purification and concentration steps should also be prioritized as they can create bottlenecks that result in delays, lower yields, and compromised product quality.

In this checklist, we explore how next-generation technologies purpose-built to improve purification and concentration efficiency can help—including the different types of Asahi Kasei Bioprocess equipment and components used to achieve these techniques—and offer considerations for adding them to your facility.

Purification **Equipment & Components**

Asahi Kasei Bioprocess offers various equipment and components to enhance the oligo purification process:

- CURSIV™ LC Systems
- CURSIV™ DAC & DAC ERGO LC Columns

CURSIV™ LC Systems

Liquid chromatography (LC) systems separate, identify, and quantify components in a liquid mixture. They are crucial in purification as they separate oligos from impurities and undesired byproducts to produce a highly pure and concentrated final product. **At Asahi Kasei Bioprocess, our engineers realize that no "one size fits all" LC system exists, so we developed a few options to fit your facility's particular processes.**

CURSIV™ MPLC Systems

For over a decade, Asahi Kasei Bioprocess has manufactured 20 bar **CURSIV™ MPLC Systems**, ideal for purifying mid-sized therapeutics like oligos using 15 to 40 µm chromatography media. These systems handle higher backpressures without the expense of traditional preparative HPLC, offering flexibility for ion exchange and reverse-phase gradient elutions.

CURSIV™ HPLC Systems

Traditional oligo purification processes using 10 to 15 µm chromatography media create high backpressures (70 to 100 bar) and require linear gradients for purity. Our **CURSIV™ HPLC Systems** handle these pressures and offer flexible gradient options for industrial and sanitary applications.

CURSIV™ UHPLC Systems

As smaller particle sizes in chromatography become more common, new impurities are surfacing that traditional HPLC can't handle at a preparative scale. Asahi Kasei Bioprocess has responded with large-scale gradient **CURSIV™ UHPLC Systems** up to 200 bar, effectively separating challenging mixtures with shorter run times.

The CURSIV™ LC Systems are automated machines equipped with pumps, valves and sensors that monitor the product and buffer feed.

These systems ensure that everything blends exactly as required. Mixing the correct buffers with salt and other components is crucial to avoid product loss. The more optimized the process, the less product will be wasted. Additionally, the systems have a built-in controller that maintains precision and closely monitors sensors. If the buffer goes out of specification, the system adjusts by pulling in different components to ensure everything stays within tolerance.

CURSIV™ DAC & DAC Ergo LC Columns

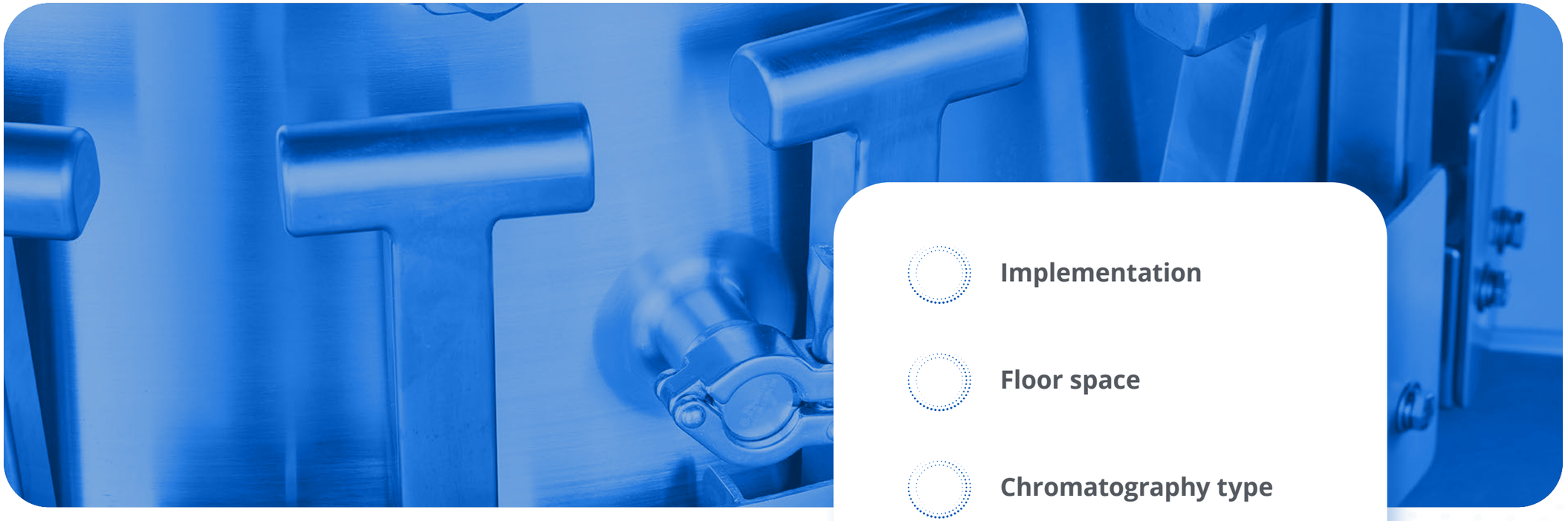
LC columns are filled with a stationary phase material where components are separated in a sample. The column's properties, such as particle size, pore size and chemistry, determine how analytes interact with it, leading to separation based on physical and chemical characteristics.

CURSIV™ DAC and DAC Ergo LC Columns transform the way chromatography columns are handled in oligosynthesis workflows thanks to their ergonomic design and advanced features.

These stainless-steel vessels, tailored for efficiency and user comfort, are ideal for large-scale manufacturers. They have a movable top plate that can be adjusted based on the type of chromatography media used.

The DAC ERGO LC Column features hydraulic legs that simplify column changeovers by allowing for easy product transfer onto a cart after completion.

This hydraulic system reduces labor, minimizes product changeover time, and enhances operator safety.



Purification **Checklist**

Follow this checklist to produce optimal results during oligo purification. These are the key factors that influence your purification process:

- Implementation
- Floor space
- Chromatography type
- Pressure
- Explosion-proof rating

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Implementation

When implementing a new purification system, ensure compatibility and communication between different pieces of equipment so they can operate in sync. For instance, if you have synthesis equipment from one manufacturer and chromatography equipment from another, they should still be able to communicate and keep collective batch records, even if they use different software. Software connectivity with your Programmable Logic Controller (PLC) is especially important for data management.

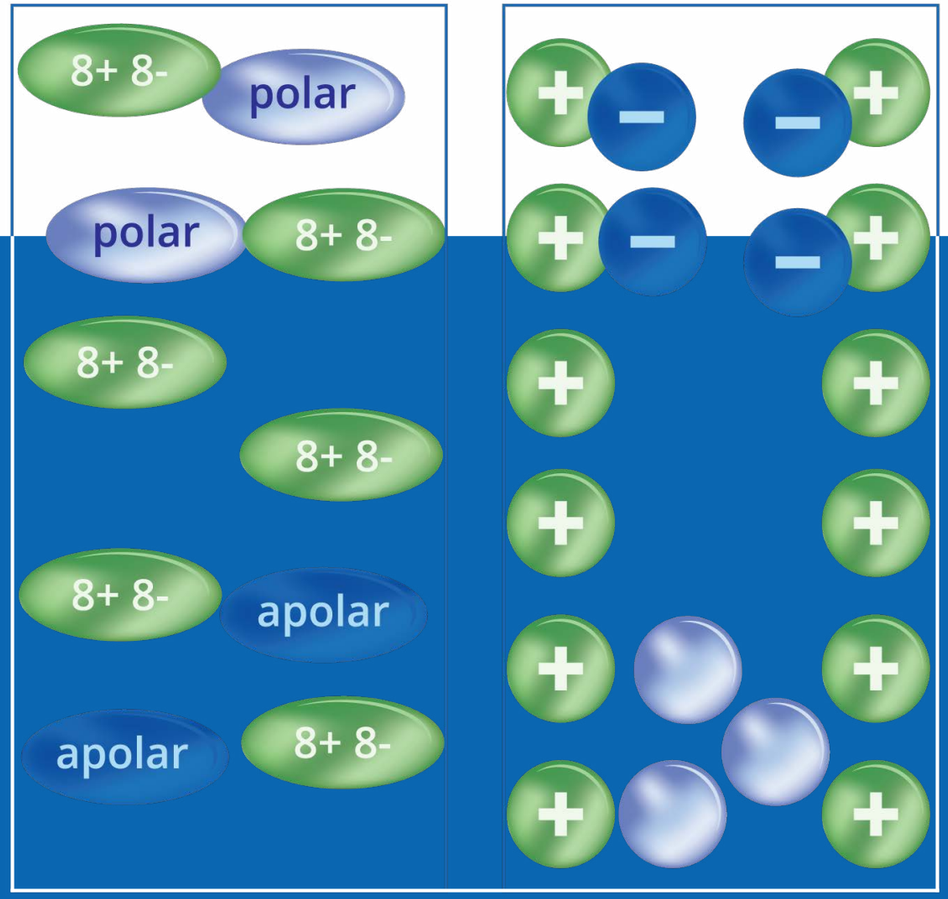
Chromatography Type

Before you choose an LC system, consider the type of chromatography you will use. Oligo purification typically uses one of the following chromatography types:

- **Reverse-phase chromatography:** In this method, the stationary phase is non-polar, while the mobile phase is polar. Oligos with longer sequences and higher purities are typically purified using reverse-phase chromatography. This method separates oligos based on their hydrophobicity, with more hydrophobic molecules eluting later.

- **Ion-exchange chromatography:** This technique separates molecules based on their charge. An ion-exchange resin with either positively or negatively charged groups is used as the stationary phase. Oligos are separated based on their net charge, with negatively charged oligos binding to positively charged resin and vice versa. Elution is achieved by changing the pH or ionic strength of the mobile phase.

Before you choose an LC system, consider the type of chromatography you will use. Oligo Reverse-phase chromatography focuses on hydrophobic interactions, and ion-exchange chromatography focuses on charge interactions. The choice between these methods depends on factors such as oligo length, purity requirements and the specific properties of the target oligo.



Reverse Phase **Ion Exchange**

Floor Space

Another critical aspect to consider when selecting equipment is floor space or footprint because you want to ensure everything will properly fit within your facility. When it comes to the actual equipment selection, the type of chromatography system plays a significant role in space requirements.

Explosion-Proof Rating

When setting up purification equipment in the same room as synthesis equipment, it's crucial for the purification system to be rated as explosion-proof. In Europe, this is known as ATEX certification, while in the US, it's referred to as a Class 1, Division 2 (C1D2) rating. This rating ensures that all materials and components used in the equipment are safe to use in a hazardous environment.

Pressure

Overpressurization or insufficient pressure can adversely affect the purification process. Your purification equipment should be robust enough to handle consistent pressure levels across each batch, minimizing fluctuations in product quality. This is especially vital for contract manufacturers aiming for consistent results with every run.

The following factors all contribute to maintaining the optimal pressure and ensuring a smooth chromatography process:

- **Vessel size**
- **Material composition**
- **Pump quality/quantity**
- **Valve types**
- **Automation capabilities**
- **Sensor utilization (including pH, conductivity, and UV sensors)**

CURSIV™ LC Systems and CURSIV™ DAC & DAC ERGO LC Columns can be customized for hazardous area operation as needed.

Concentration **Equipment**

To increase the concentration of oligo samples, you need equipment that removes excess solvents and contaminants. Asahi Kasei Bioprocess offers ultrafiltration/diafiltration tangential flow filtration (TFF) systems that help to create highly concentrated oligo samples.

VANTIJ™ Ultrafiltration/Diafiltration TFF Systems

The **VANTIJ™ Ultrafiltration/Diafiltration TFF System** is tailor-made for oligo manufacturing environments with hazardous conditions. While TFF systems are common in biopharmaceutical development, this UF/DF TFF System is unique, designed from scratch to integrate into oligo manufacturing processes seamlessly.

Equipped with explosion-proof components and regional certifications, the UF/DF TFF System assures reliable operation even in challenging environments. It's designed for flexibility, supporting widely available cassette-style filters with a total area of up to 20 sqm.

Advanced Controls

The **UF/DF TFF System** offers advanced process controls to ensure precise and accurate results. Every pump is monitored and controlled by an independent Coriolis mass flowmeter to ensure consistent flow rates and total volume delivery. Transmembrane pressure can be used to control pump speeds, minimizing the filtration time for a given batch.

Free to Customize

Although the standard UF/DF TFF models work well for most applications, Asahi Kasei Bioprocess recognizes that your synthesis processes or multi-product needs may require a personalized approach. Our skilled engineers and designers are prepared to develop a custom solution using our foundational designs. Options for customization include integrated filter integrity testing and a bottom-mounted agitator for the retentate tank.

Concentration **Checklist**

Oligo concentration can be challenging unless you have the right equipment. Use this checklist to ensure you choosea concentration system that meets your needs:

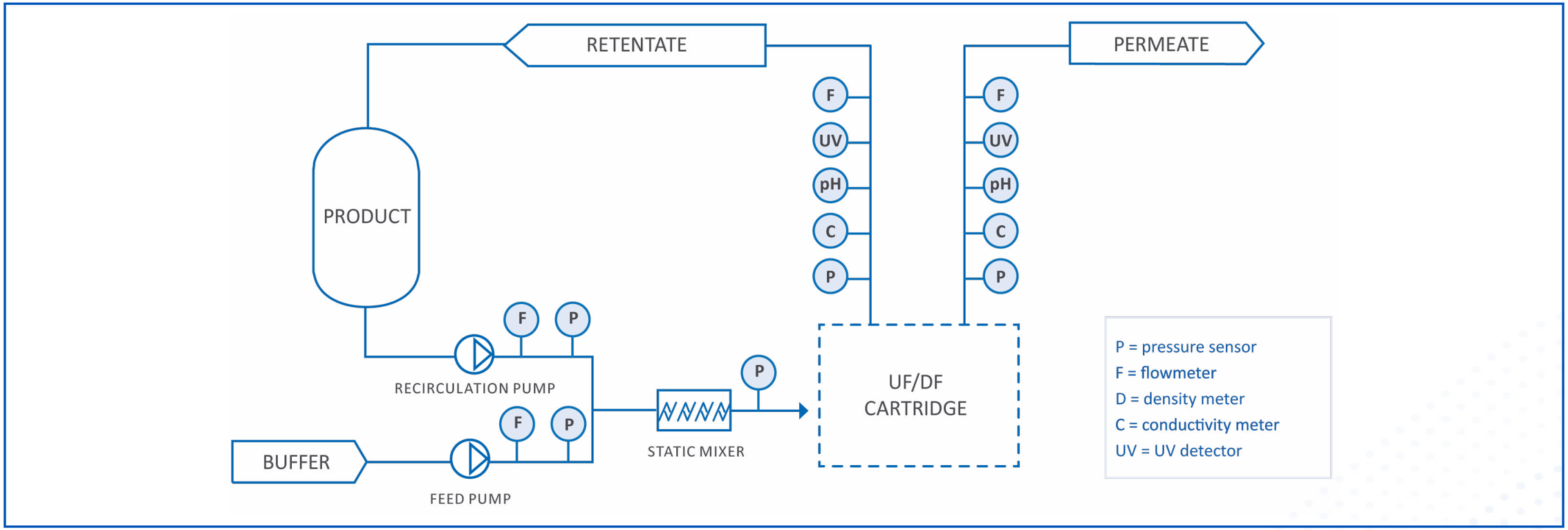
- **Implementation**
- **Floor space**
- **Filters**
- **Explosion-proof rating**

Implementation

It is crucial for success to utilize oligo concentration equipment that can effectively communicate with other manufacturing equipment. Seamless integration and data exchange among systems guarantees precise process control, dependable data management and a smooth workflow. This interoperability not only boosts efficiency but also reduces errors and optimizes the overall performance of your oligo concentration processes.

Floor Space

Properly sized equipment ensures efficient utilization of your manufacturing area, minimizing clutter and optimizing workflow. Additionally, equipment that matches your space constraints helps create a safer and more organized working environment that facilitates smooth operations and reduces the risk of accidents or disruptions.



Filters

The choice of filters can influence your UF/DF TFF system's pressure capacity, sensor compatibility and overall efficiency. Using filters compatible with the equipment's design and specifications ensures optimal performance, reliable operation and accurate monitoring during the concentration process. The **VANTIJ™ UF/DF TFF System** uses cassette-style filters that are readily available.

Explosion-Proof Rating

Make sure you use a UF/DF TFF system with an ATEX or C1D2 rating to implement it alongside your other synthesis equipment in the same room. This safety measure also protects your team, equipment and environment from hazards associated with the concentration process.

The VANTIJ™ Ultrafiltration/ Diafiltration TFF System has an explosion-proof rating.

Common Issues **& Troubleshooting**

Oligo purification and concentration processes can be complex because they require precise control over multiple variables such as reagent purity, flow rates, pressure, and temperature. Any deviations can lead to significant variations in yield and purity.

To keep your operations running, here are actionable tips for overcoming common equipment issues and steps for troubleshooting your processes.

Common Oligo Purification & Concentration Equipment Challenges

Even high-quality purification and concentration equipment and components can run into trouble during oligo manufacturing. Here are common challenges you may encounter during purification and concentration, along with actions you can take to overcome them.

Time Between Batches

When you work with purification equipment, the time required between batches often emerges as a significant bottleneck. This downtime not only hampers overall productivity but also increases operational costs and delays project timelines. Addressing this issue by streamlining the purification process to minimize idle periods is crucial.

Optimizing the flow rate, enhancing filter efficiency, and automating various stages can significantly reduce this bottleneck. By making these processes as swift and efficient as possible, you can achieve faster turnaround times, higher throughput, and ultimately, more cost-effective operations.

Sensor Calibration

Accurate sensors ensure that measurements of flow rates, pressure and temperature are reliable, directly impacting the quality and consistency of the purification and concentration processes.

Asahi Kasei Bioprocess recognizes this critical need, so we thoroughly calibrate sensors before shipping our equipment to guarantee initial accuracy. We also offer ongoing support through our 847 Continuum Program™, which includes recalibrating and verifying sensor accuracy. This proactive approach helps maintain optimal performance and reliability.

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Troubleshooting Tips

When encountering issues such as low oligo yield, impurities or other inefficiencies in purification and concentration processes, several troubleshooting steps can be instrumental in identifying and resolving the problems.

- Ensure all reagents and solvents are of high purity.
- Verify the proper functioning and alignment of the chromatographic columns.
- Ensure the equipment is properly calibrated.
- Review and optimize protocol parameters, such as flow rates, gradient profiles, and elution times.
- Check for blockages or leaks in the system that may disrupt flow and lead to inconsistencies.
- Maintain and clean the equipment to prevent contamination and ensure optimal performance.

If problems persist, consulting with Asahi Kasei Bioprocess for support and potential recalibration services can provide further insights and solutions.

Our **847 Continuum Program™** offers support, training, maintenance and parts and consumables to keep your oligo production running smoothly. Whatever your requirements, we work with you to configure the right plan, schedule, and pricing.

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The right support for purification and concentration equipment can improve efficiency and keep your oligo production on schedule.

Conclusion

The rising demand for oligos has prompted biomanufacturers to pursue advanced purification and concentration technologies to boost yield and quality.

To address these needs, seek to add the most cutting-edge LC systems, LC columns and ultrafiltration/diafiltration TFF systems to your facility. These technologies are designed to streamline and improve oligo manufacturing workflows.

The provided checklists and troubleshooting tips offer actionable guidance for implementing and optimizing these systems, ensuring that biomanufacturers can maintain high productivity and quality standards in their oligo production. With these advancements, the industry is well-positioned to meet the projected market growth and continue supporting innovations in scientific research and medical therapies.

Asahi Kasei Bioprocess offers innovative equipment and components for oligonucleotide purification and concentration, including the CURSIV™ MPLC System, CURSIV™ HPLC System, CURSIV™ UHPLC System, CURSIV™ DAC and DAC Ergo LC Columns, and the VANTIJ™ Ultrafiltration/Diafiltration TFF System. Our products are built for you to meet all your needs. To learn more about our products, **CONTACT US**.

VANTIJ™ CURSIV™

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Contact Information

North and South America

Glenview, IL, USA
Tel: +1-847-556-9700
Fax: +1-847-556-9701

Email: info.us@ak-bio.com

Europe

Cologne, Germany
Tel: +49-221-995007-59
Fax: +49-221-9950077-59

Email: info.eu@ak-bio.com

India and Southeast Asia

Mumbai, India
Tel: +91-22-6710-3962
Fax: +91-22-6710-3979

Email: info.jp@ak-bio.com

East Asia and Oceania

Glenview, IL, USA
Tel: +1-847-556-9700
Fax: +1-847-556-9701

Email: info.jp@ak-bio.com

China

Shanghai, China
Tel: +86-(0)21-6391-6111
Fax: +86-(0)21-6391-6686

Email: info.jp@ak-bio.com