AsahiKASEI BIOPROCESS

9 Essential Oligosynthesis Component Needs for 2023

Steve Foy
Manager, Products and Brand Strategy at Asahi Kasei Bioprocess America

Steve received his Bachelor of Arts (B.A.) in Advertising from Marquette University and Master of Science (M.S.) in Integrated Marketing Communications from Northwestern University.

The chemical process of synthesizing oligonucleotides is called oligosynthesis. It involves assembling individual nucleotides – the building blocks of nucleic acids – into a desired sequence.

Oligosynthesis components are in high demand as the need for more efficient and scalable ways to produce oligonucleotides continues to grow. But what kind of systems and columns should your production facility have?

Before we jump headlong into oligo components, let's do a short review of oligonucleotides and the oligosynthesis process. If you're already an expert, feel free to skip this part and go straight to component needs below.

WHAT ARE OLIGONUCLEOTIDES?

Oligonucleotides are short single-stranded DNA or RNA molecules that contain a few nucleotides. They are usually synthesized in a laboratory and used as probes to identify and analyze genes or to produce gene fragments for research.

Scientists and researchers also use oligonucleotides in biotechnology and medicine, such as gene therapy, diagnostics, and drug delivery. For example, oligonucleotides can serve as:

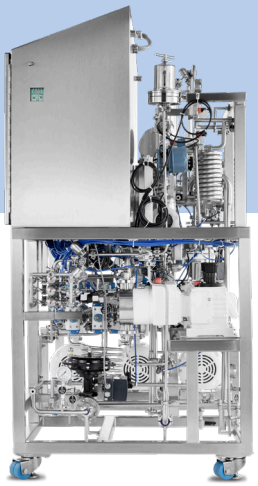
- "Gene silencing" agents to reduce the expression of genes involved in disease
- Immunomodulators to stimulate or suppress the activity of the immune system
- Inhibitors of cancer-causing genes and deter the growth and spread of tumor cells

But how are oligonucleotides made? The answer is oligosynthesis.

THE OLIGOSYNTHESIS PROCESS

The chemical process of synthesizing oligonucleotides is called oligosynthesis. It involves assembling individual nucleotides – the building blocks of nucleic acids – into a desired sequence.

The researcher determines this sequence and typically encodes a specific gene or region of interest. Then they can use the synthesized oligonucleotide for various genetic engineering and research applications, including gene therapy, gene editing, and diagnostic testing.



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Oligosynthesis is a relatively new technology that has enabled the rapid and efficient synthesis of short DNA sequences. It has revolutionized biotechnology and has a wide range of applications, from academic research to biomedicine. And because oligonucleotides have so many essential uses, they are in high demand. In 2021, the global oligosynthesis market was valued at USD 7.3 Billion. By 2028, those numbers are expected to reach USD 22.5 Billion.

But manufacturing oligonucleotides at a commercial scale requires dedicated components for each part of the process. Here's what you'll need.

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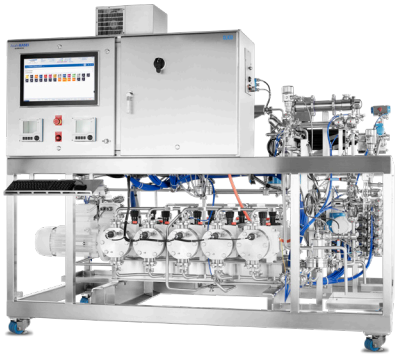
9 MUST-HAVE COMPONENTS FOR SUCCESSFUL OLIGOSYNTHESIS

While there are many different types of components used in oligosynthesis, we want to highlight nine of the key pieces that can help you reduce manufacturing costs and increase product yields:

1. Oligosynthesizer
2. Synthesis columns
3. Cleavage & deprotection (C&D) system
4. Purification system
5. Purification columns
6. Slurry preparation system
7. System control software
8. Buffer management system
9. Analytical instruments



1. OLIGOSYNTHESIZER

An oligosynthesizer is an innovative tool that automates the process of producing oligonucleotides quickly and reliably. By producing large numbers of oligonucleotides, researchers can rapidly identify and synthesize novel molecules that may have therapeutic properties. Additionally, the oligosynthesizer can be used to produce DNA fragments for use in the production of gene therapies.

2. SYNTHESIS COLUMNS

These stainless-steel columns are essential because they can synthesize a wide variety of oligonucleotides, including those with different lengths, sequences, and modifications.

Synthesis columns retain a well-packed bed of solid support during the turbulent and lengthy synthesis process. They also ensure an equal distribution of phosphoramidites and reagents to all beads in the packed bed.

Each column fills with a buffer solution containing a detergent, such as sodium dodecyl sulfate, which helps to keep the oligonucleotides in the solution. The column is then heated to the desired temperature, allowing the oligonucleotides to bind to the solid supports. The desired oligonucleotides are then eluted from the column while the impurities stay.

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Two popular types include:

1. **Axial compression synthesis (ACS) columns:** Flow-through synthesis columns that are typically used in high-throughput applications and are especially useful for synthesizing large quantities of oligonucleotides.
2. **Static compression synthesis (SCS) columns:** Active flow synthesis columns that are often used for fixed bed height applications using swellable supports.

3. CLEAVAGE & DEPROTECTION (C&D) SYSTEMS

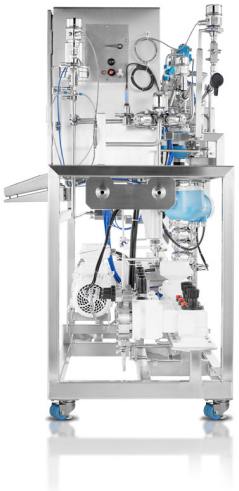
Cleavage and deprotection (C&D) systems are one of the most important components of the process. They remove the oligonucleotide or polypeptide from the solid support and then extract the protecting groups from the oligonucleotide or polypeptide chain.

These systems can handle both DNA and RNA processes, and most can automate the cleavage and deprotection steps to boost synthesis capacity.

4. PURIFICATION SYSTEM

The purification system works by taking a mixture of oligonucleotides and separating them into their components. This is done by passing the mixture through a series of filters and columns.

The filters are designed to capture the desired oligonucleotide, allowing the undesired material to pass through. Then, the columns separate the desired oligonucleotide from the other components of the mixture. This establishes the purity of the oligonucleotide.



Two popular kinds of purification systems are:

- **Medium pressure liquid chromatography (MPLC) systems**: These separate and identify components in a mixture. MPLC systems use a pump to force a solvent through a column filled with stationary phase material.
- **High performance liquid chromatography (HPLC) systems:** These separate, identify, and quantify compounds in complex mixtures. HPLC systems consist of a pump, injector, column, detector, and data processing and storage device.

5. PURIFICATION COLUMNS

Purification columns utilize solid phase extraction (SPE), a technique in which a sample is loaded onto a column packed with an inert stationary phase. This stationary phase is usually made of silica or polystyrene and is designed to be highly selective, allowing only certain components of the sample to pass through. At that time, the desired components wash away while the column retains the contaminants.

When used for oligonucleotide purification, the column's stationary phase is typically composed of silica and is specially designed to be highly selective for oligonucleotides. It also minimizes the loss of samples during the purification process.

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The column is then loaded with a sample containing the desired oligonucleotides and subjected to a series of washing steps to remove any contaminants. A piston drives the columns to add compression. Finally, the desired oligonucleotides are eluted off the column, leaving any contaminants behind.

6. SLURRY PREPARATION SYSTEM

Slurry preparation systems allow equipment operators to quickly and easily prepare the media that's used in the purification column to properly purify the oligonucleotide product from waste components.

Proper mixing and removal of "fines" from your purification resins are crucial to a successful column packing. The media is added to the system from supplied totes, jugs or drums. Once in the SPS, a series of steps are performed to fully saturate the media, remove any fines present due to handling, and ultimately creat a homogenized slurry to pack the purification column.

Slurry prep systems assure consistent outcomes by eliminating the potential for disparities in approach by operators and greatly reducing the potential for human error. Improvements to the column packing result in higher yields, better throughput and more precise separation of the target oligo from any undesired compounds in the product stream.

Slurry Prep systems assure consistent outcomes by eliminating the potential for disparities

7. SYSTEM CONTROL SOFTWARE

An oligonucleotide synthesis automation platform is computer software that operators can leverage to quickly and accurately synthesize a large number of oligonucleotides of varying lengths and sequences. This can be especially useful for genome-wide analysis and other large-scale projects. Additionally, it can be used to efficiently synthesize a specific oligonucleotide for use in mass production.

The ideal oligonucleotide synthesis software platform is

- Integrated into the oligosynthesizer
- User-friendly and flexible
- Capable and robust
- Able to manage large amounts of data
- Universally compatible

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8. BUFFER MANAGEMENT SYSTEM

An automated buffer management system is designed to maintain the pH of a solution within a narrow range. An ideal buffer system can blend a precise mixture of buffer concentrates and salts that helps stabilize the pH of the solution and prevent degradation of the oligonucleotides. The most commonly used buffers for oligonucleotides are Tris-HCl, phosphate, and glycine. Tris-HCl is a strong acid-base buffer that's generally used at pH 7.5-8.5. Phosphate and glycine buffers are weaker acid-base buffers that are typically used at pH 8-9.

9. ANALYTICAL INSTRUMENTS

Analytical instruments allow scientists and researchers to study the structure and function of DNA and RNA molecules, as well as to identify and characterize genetic markers.

Some of the most common instruments include:

- Capillary electrophoresis (CE) instruments are used to separate, detect, and quantify oligonucleotides, and they can identify specific sequences of DNA and RNA.
- Mass spectrometers identify large molecules such as proteins and polysaccharides, and they can also analyze oligonucleotides.
- DNA microarrays detect and analyze genetic expression and can identify single-nucleotide polymorphisms (SNPs).

CONCLUSION

As oligosynthesis continues to heat up, so too will demand for the components required to meet the challenge. This paper was about providing a foundational understanding of what systems you'll need to make room for in your production facility. Your next steps should focus on finding an equipment partner that can help you achieve your production goals for 2023 and beyond.

Need oligosynthesis help? Asahi Kasei Bioprocess offers state-of-the-art oligosynthesis equipment that's built to meet your spatial, performance, and cost needs. Start by talking to one of our experts.

Questions?

Email me at steve.foy@ak-bio.com