以下注册商标专用权，归江苏汉邦科技股份有限公司所有
Bonpure®、Benetnach®、Ceres®、Dubhe®、Hanbon®、Kenase®、Libra®、Megres®、Phecda®、Newstyle®、Taurus®、Nucifera®、Hedera®

技术规格如有改变，恕不另行通知，本公司保留最终解释权和修改权 2022年8月印制

精粹色谱科技 致力人类健康

汉邦科技
Hanbon Sci.& Tech.

# OVERALL SOLUTION FOR OLIGONUCLEOTIDE PRODUCTION

## PRODUCT MANUAL

- Solid-phase synthesis
- Purification
- Filtration
- Oligonucleotide Synthesis Column

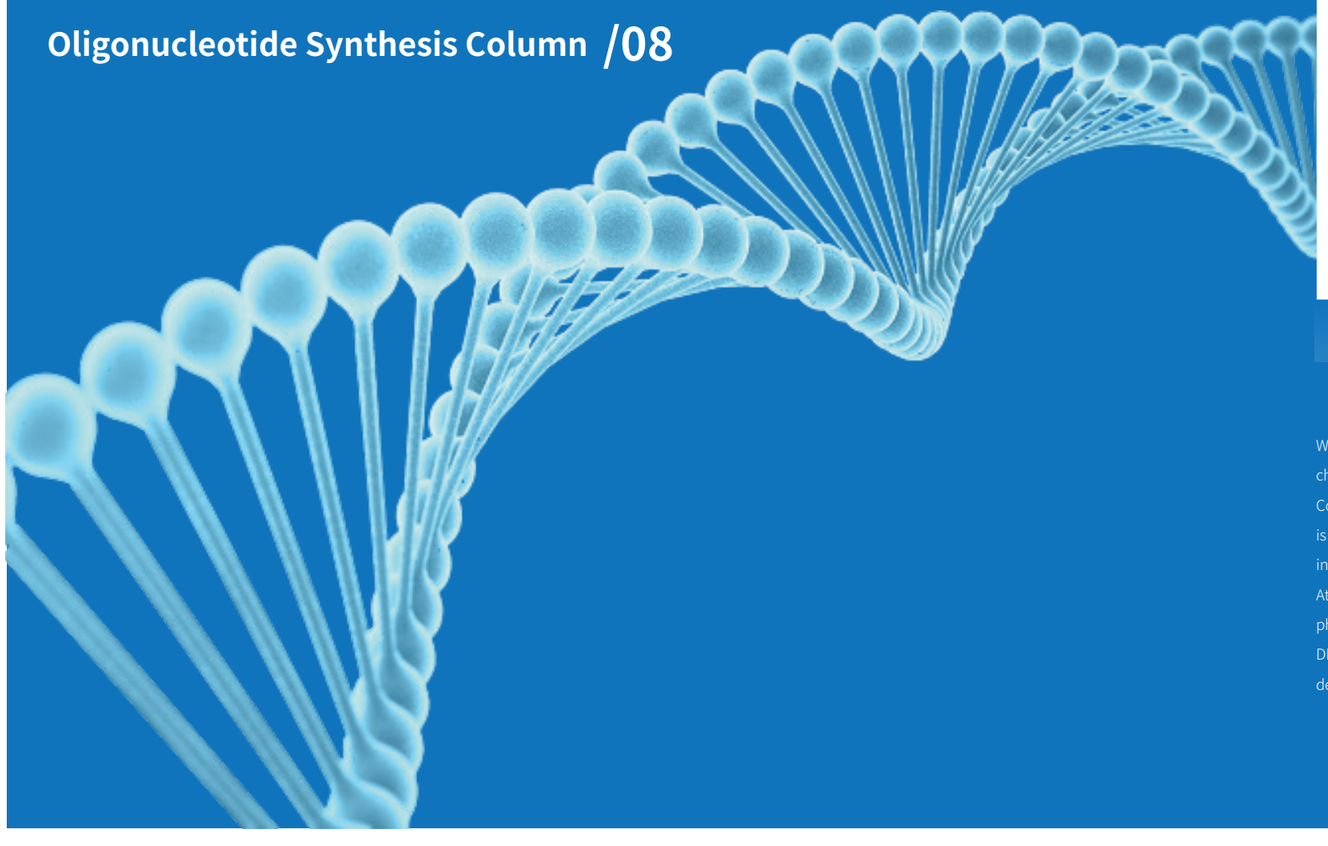
江苏汉邦科技股份有限公司
Jiangsu Hanbon Science & Technology Co., Ltd.

江苏汉邦科技股份有限公司
Jiangsu Hanbon Science & Technology Co., Ltd.
江苏省淮安经济技术开发区集贤路1-9号
+86 517 83706900
+86 517 83706903
globalbusiness@hanbon.com.cn
www.jshanbon.com

汉邦公众号

## >>> CONTENTS





Hanbon Sci. & Tech.
精粹色谱科技 致力人类健康

## 1 >>> OVERVIEW

As a representative of nucleic acid drugs, mRNA vaccines have shined in the sudden COVID-19 epidemic, showing good protection. Another branch of nucleic acid drugs: oligonucleotide drugs, has also received unprecedented attention.

Oligo drugs mainly include antisense nucleic acid (ASO), small interfering nucleic acid (siRNA), microRNA (miRNA), nucleic acid aptamer (Aptamer), etc. Compared with traditional small-molecule chemical drugs and biological macro-molecular antibody drugs, nucleic acid drugs can intervene from the source, inhibit the expression of disease-related genes, or introduce genes that can express normal proteins to make up for the lack of functional proteins. It has “cure both the symptoms and root” feature. Coupled with the advantages of simple design, short development cycle, long-acting effect, rich targets, and strong specificity, nucleic acid drugs have shown great commercial value in the fields of genetic diseases, metabolic diseases, and cancer.

Traditional small-molecule chemical drugs and macro-molecular antibody drugs are leading the first and second waves of the pharmaceutical industry, then we have absolute reason to believe that nucleic acid drugs will lead the third wave of the pharmaceutical industry.

**SMALL MOLECULE DRUGS**

The most common disease treatment drugs, usually including small molecular compounds that act on cell cycle, enzymes, ion channels, etc.

It is easy to produce and can be administered orally

>>>

**ANTIBODY DRUGS**

Drugs that can target pathogenic cell proteins or signal pathways can accumulate or release effective ingredients at the target site

✓ Smaller molecular drug proteins have stronger affinity, and the number of target proteins that can become drugs is limited

× High production cost, injection, action site is only extracellular or cell surface

>>>

**NUCLEIC ACID DRUG**

Introduce exogenous nucleic acid drugs into target cells to silence or compensate for diseases caused by defects and abnormal genes to achieve therapeutic purposes

✓ To treat diseases at the transcriptional level, not restricted by non-productive proteins, and has high efficiency, strong specificity and multiple action sites

× It is necessary to combine advanced drug delivery system to achieve targeting and improve drug safety

Figure 1: Evolution trend of therapeutic drugs

What we usually call small nucleic acid drugs, or oligonucleotide drugs, are short chains of nucleic acids composed of a dozen to dozens of nucleotides in tandem. Combined with the current FDA-approved small nucleic acid drugs, the main focus is on ASO drugs and RNAi drugs, which achieve the purpose of treating diseases by interfering with the expression of target genes.

At present, the synthesis of DNA/RNA can be carried out by methods such as liquid phase synthesis, biosynthesis and solid phase synthesis, but the commercial DNA/RNA synthesis is still dominated by solid phase synthesis, which undergoes deprotection, coupling, oxidation (sulfo), blocks and other links.

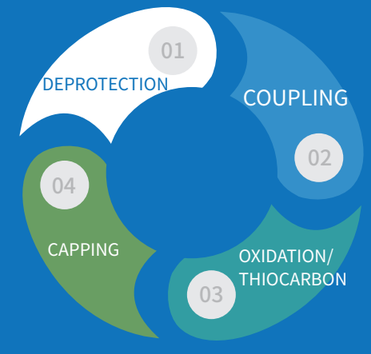



Figure 2: Principle of oligonucleotide synthesis

# 2 Oligonucleotide Solid-phase Synthesis

## Lab-scale oligonucleotide synthesis

Bio-Oligo equipment is an efficient, fast and reliable oligonucleotide synthesis equipment, independently developed by Hanbon Sci & Tech Co. Ltd. At present, this series mainly has two types. Bio-Oligo 10 is mainly used for synthesis in the range of 1 μmol to 50 μmol, while Bio-Oligo 100 can synthesize DNA/RNA within 50 μmol to 9 mmol. The equipment adopts modular design and intelligent software. Combined with synthesis columns of different specifications, it can synthesize certain amount of DNA/RNA, this system can be used for clinical research, nucleic acid drug development and synthesis of molecular diagnostic probes.

Figure 3：Laboratory oligonucleotide synthesis

### Synthesis scale

| Item | Synthesis scale |
|---|---|
| Bio Oligo 10 | 1umol~50umol |
| Bio Oligo 100 | 50umol~9mmol |

### Configuration parameters (Bio-Oligo 100)

| | | | |
|---|---|---|---|
| Power supply | 220V±5% AC | Synthesis scale | 50μmol-9mmol |
| Wattage | 900W | Solvent Compatibility | Deprotection reagents, BTT, thio reagents, etc. |
| Weight | 55kg | Column reactor | 1 |
| Dimensions | 450*570*570mm | Amidite bottle | Standard 8, expandable to 18 |
| Operating temperature | 10~40℃ | Single cycle time | ＜30min |
| Humidity | 30% | Synthetic efficiency | ＞99% for DNA<br>＞98% for RNA |

## Pilot-scale oligonucleotide synthesis

Bio Oligo Pro 400 pilot-scale nucleic acid synthesizer is an equipment independently developed by Hanbon Technology Co., Ltd. for pilot-scale nucleic acid synthesis. The equipment can be used for synthesis of DNA/RNA in the range of 4mmol to 60mmol. Also, for the synthesis of partially modified DNA/RNA, the recommended synthetic sequence is 20bp~40bp. The equipment adopts explosion-proof design, which can meet the general GMP production requirements. It has a compact design and cover a small area, and can be used for pilot scale-up production to meet the needs of batch production of hundreds of grams. The advent of this equipment has solved the gap in this field of domestic equipment to a certain extent, and alleviated the long-term monopoly of imported equipment in this field.

Figure 4：Pilot-scale oligonucleotide synthesis





### Configuration parameters (Bio Oligo Pro 400)

| | | | |
|---|---|---|---|
| Infusion Pump | Piston pump, stainless steel, pump head self-cleaning function | Protective gas pressure | Nitrogen/Argon, 0.3~0.35bar |
| Flow rate range | 0~600ml/min | UV detector | Four-channel UV detector, wavelength range 200~800nm, Accuracy±1nm |
| Synthetic scale | 4mmol~60mmol | Conductivity detector | Detection range 1uS/cm~500mS/cm |
| Single entry quantity | 11 (standard configuration) | Dimension | 1426x1037x1935 mm (L x W x H) |
| Number of Column Reactors | 1 | Power supply pressure | 220V±5%AC |
| Maximum operating pressure | 15 bar (218psi) | Power | 1500W |
| | | Pipe material | PTFE, PEEK |

# 3 Purification

After the initial sample is ammonolyzed, it will go through a water bath time of about 10 hours, then the product is cooled to room temperature, collected by filtration, rinsed with ultra pure water three times, the filtrate is collected, and the volume is constant to obtain the crude product. Next, the concentration of the crude product and the purity of the crude product are tested to determine the quality of this batch of samples. If the purity of the initial sample meets the requirements, further purification work is generally required to meet the purity requirements for the raw material drug. In the purification process, due to the differences in the purification process paths, there are generally two methods to choose from, reversed-phase chromatography and ion-exchange chromatography, and pharmaceutical companies themselves will choose one or two of them for purification based on their own advantages.

## > Purification - Reverse-Phase Chromatography

### V Laboratory scale HPLC system

The high-performance liquid chromatography system is the equipment that will be used when reversed-phase chromatography is used for purification, and the use of C18 packing material can meet the purification work of laboratory-scale oligo crude products. The high performance liquid chromatography system has also been widely used in the screening of active substances in the research and development of natural medicines, microbial fermentation medicines, peptide medicines, and chemically synthesized medicines. It can prepare milligram-level to gram-level active substances to meet the needs of purification process development.

Figure 5：Laboratory HPLC system


Figure 6：Industrial preparative liquid chromatography system

### V Industrial scale preparative liquid chromatography system

In the pilot test and industrial production stages of the purification process, industrial preparative liquid chromatography systems can be used for separation and purification. As an industrialized separation and purification equipment, the CS-Prep industrial preparation liquid chromatography system has the characteristics of high throughput, rapidity, automation, and explosion-proof. It is widely used in the separation and purification of medicine, chemical industry, plant extraction, food and other fields, effectively improving Production efficiency, degree of automation and product purity and yield.

CS-Prep industrial preparation liquid chromatography system consists of infusion part, sample injection part, detection part, fraction collection part, control and data processing part. The overall design of the equipment complies with relevant regulations such as GMP, cGMP and FDA, and can realize processes such as balance, sample loading, washing, elution, automatic collection, and online cleaning, and can provide verification documents that comply with relevant regulations such as GMP.





### V Configuration parameters

| | | | |
|---|---|---|---|
| Item | CS-Prep 200 | Inner diameter of cylinder (mm) | 200 |
| Maximum flow rate of infusion pump (L/h) | 200 | Column length (mm) | 680 |
| Column | DAC 150~200 | Inner wall roughness (um) | ⩽0.2 |
| Material | SS316L | Effective filling height (mm) | ⩽450 |
| Maximum working pressure (bar) | 100 | Maximum pressure (bar) | ⩽100 |
| Explosion-proof grade | ExdIIBT4 | Packing method | Down |

## > Purification -Ion Exchange

### V Bio-Lab laboratory chromatography system

Bio-Lab™ laboratory chromatography system is an efficient, fast and reliable automatic chromatography equipment independently developed by Hanbon, applied to the rapid purification of bio-molecules such as peptides and nucleic acids etc. from microgram to gram. The system adopts modular design and intelligent software, and can meet the purification needs of various biological macro-molecules in the laboratory with different specifications of chromatography columns.


Figure 7：Oligonucleotide purification instrument

### V Configuration parameters

| Model | Bio Lab 30 | Bio Lab 100 | Bio Lab 300 |
|---|---|---|---|
| System pump | Binary plunger piston pump, material: PEEK, titanium alloy or 316L stainless steel. It has good biocompatibility, self-flushing function of pump head, preventing pollution and crystallization,with electronic pressure pulsation compensation, providing excellent gradient accuracy and repeatability forlaboratory chromatography system to ensure the purification reproducibility. | | |
| Flow rate (ml/min) | 0~30 | 0~100 | 0~300 |
| System pressure | 0~5MPa (Customized) | | |
| Flow rate accuracy | ±0.5% | | |
| Gradient mode | Linear, isocratic, and stepwise elution gradients, gradient ratios can be modified online | | |

### Bio-Pro ® Pilot / Process Chromatography System

In the lab stage, the crude synthesized nucleic acid can be purified by using Bio-Lab nucleic acid purification instrument combined with ion exchange chromatography. Lab chromatography system is more suitable for the purification of crude products from milligram to gram. The future process amplification will need to use pilot scale or industrial scale chromatographic system.

Bio-Pro® automatic chromatography system is suitable for pilot and industrial scale production of biopharmaceutical purification process. The system is designed as integrated module according to ASME BPE and GMP, and can be configured in various ways according to the specific requirements of customers. The system can manually or automatically realize balance, sample loading, washing, elution, automatic collection, online cleaning and other processes, and can provide verification documents met GMP requirements.

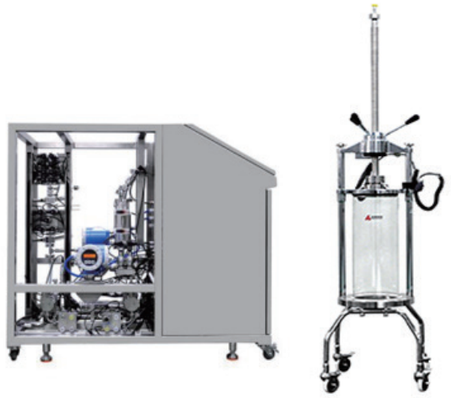


Figure 8：Bio Pro ® chromatography system + Manual Chromatography Column

### Configuration parameters

| Item | Bio Pro 180 | Inner Diameter(mm) | MCC-200 |
|---|---|---|---|
| Infusion Pump | Sanitary quaternary diaphragm pump | Column Height (mm) | 500/750/950 |
| Flow Range (L/h) | 1~180 | Operation Method | Manual |
| Flow Accuracy | 1% or 1.5L/h | Pore Size(um) | 10/23(PP) or 10/20(SS316L) |
| Material | SS316L | Cylinder Material | Schott finished glass |
| System Pressure (bar) | 6 | Maximum Pressure (bar) | 6 |





## 4 Filtration

### Laboratory scale automatic tangential flow filtration system

Bio-Lab TFF automatic tangential flow filtration system is an easy-to-use automatic UF/DF system, suitable for the research and development of antibodies, vaccines, nucleic acid drugs, etc. in bio-pharmaceuticals and small-scale harvesting, clarification or concentration, and liquid exchange process and so on. It adopts a fully automatic intelligent design, automatically controls TMP, and can realize functions such as automatic concentration, equal volume filtration, automatic collection, and automatic data recording. There are currently two models available, Bio-Lab TFF 18/180 meets the needs of different membrane materials.

Figure 9：Laboratory scale TFF

### Configuration parameters

| Model | Bio Lab TFF 18 | Bio Lab TFF 180 |
|---|---|---|
| Circulation pump | Peristaltic pump | Diaphragm pump |
| Circulation pump Flow rate | 0~300ml/min | 1-180L/h flow accuracy±1% |
| Rehydration pump | Peristaltic pump | Peristaltic pump |
| Rehydration pump Flow rate | 0~300ml/min | 1-90L/h |

| Model | Bio Lab TFF 18 | Bio Lab TFF 180 |
|---|---|---|
| Maximum pressure of the system | 2 bars | 6 bars |
| Pipe material | PFA plastic hard tube | SS316L (ASME BPE) |
| Circulation tank | 500ml | 4L |
| Waste tank | / | 4L |

Figure 10：Industrial scale automatic tangential flow filtration system

### Industrial scale automatic tangential flow filtration system

Bio-TFF system is an easy-to-use automatic UF/DF system, suitable for the pilot scale and industrial scale production in biomedical industry. Bio-TFF system adopts innovative and intelligent design, which can not only improve process performance, but also achieve extremely low minimum operating volume, maximum ultrafiltration concentration multiple and optimal product recovery function.

According to customer's specific process, Bio-TFF system can be equipped with an intermediate circulation tank from 10 L to 500 L. The circulation tank and the system are separated, which does not affect the operating volume of the system. The system can realize automatic CIP, liquid concentration, equal volume diafiltration, automatic collection and other functions through manual or automatic methods, widely used in the concentration and diafiltration of small nucleic acids, monoclonal antibodies, vaccines, blood products and other biomacromolecules ideal device.

# 5 Oligonucleotide Synthesis Column

## Features

1. 316L stainless steel;
2. Specifications 6.3ml, 12ml, 24ml and 48ml are available;
3. Material of synthetic column combines biocompatibility and harsh organic conditions;

## Specifications of small synthesis column:

| Model | Volume | Inner Diameter (ID) | Column Height |
|---|---|---|---|
| SYC 6.3 | 6.3ml | 20mm | 20mm |
| SYC 12 | 12ml | 27mm | 21mm |
| SYC 24 | 24ml | 35mm | 25mm |
| SYC 48 | 48ml | 44mm | 32mm |


## Small synthesis column

1. The inner diameter of the cylinder is 35mm, and the volume range is 10ml~100ml;
2. The height of the column bed can be adjusted, which is convenient and flexible. The recommended working height is 3cm~10cm;

| Model | Inner Diameter (ID) | Column Height |
|---|---|---|
| SYC 35 | 35mm | 150mm |





## Features of medium-sized synthesis column:

1. Synthesis column specification ID 60mm, ID 100mm and ID 150mm, the maximum pressure is 2MPa;
2. The synthesis column is equipped with a distributor to ensure that the solution can be evenly distributed on the frit;
3. The synthesis column is resistant to acetonitrile, toluene and a certain concentration of dichloroacetic acid;

| Model | Inner Diameter (ID) | Column Height |
|---|---|---|
| SYC 60 | 60mm | 100mm |
| SYC 100 | 100mm | 390mm |
| SYC 150 | 150mm | 400mm |

## Features of production synthesis column:

1. The inner diameter of the cylinder is 600mm, and the maximum pressure is 2MPa;
2. The synthesis column is equipped with a distributor to ensure that the solution can be evenly distributed on the frit;
3. The synthesis column is resistant to acetonitrile, toluene and a certain concentration of dichloroacetic acid;

| Model | Inner Diameter (ID) | Height |
|---|---|---|
| SYC 600 | 600mm | 1300mm |