ACCELERATING YOUR ANTIBODY DISCOVERY

- Single B Cell Screening
- HTP Antibody Production
- VHH Antibody Discovery
- Stable Cell Line Generation
- Hybridoma Sequencing
- In Vivo Proteins and Antibodies
- Large Scale Antibody Production
- Antibody Humanization & Affinity Maturation
COMPANY PROFILE

About Biointron

Biointron, founded in 2012, is an award-winning Chinese High-tech Enterprise. We specialize in antibody expression, discovery, and optimization. Our mission is to accelerate the antibody discovery process to provide one-stop antibody discovery services. With fully automated production processes adhering to international standards, we've served 1,200+ pharmaceutical companies across more than 20 countries. Our expertise includes VHH production, hybridoma sequencing, antibody humanization, affinity maturation, and single B cell screening. Biointron has global commercialization reauthorization for ECACC's CHO-K1 cell line and meets international multicenter requirements, which has also been filed with the U.S. FDA's DMF and several partners’ projects have entered the international multicenter clinical stage.

For more information, please visit our official website at www.biointron.com or contact us at info@biointron.com.
A  SERVICES

High Throughput Antibody/Protein Expression

Bispecific Antibody (BsAb) Production

Large Scale Production

Hybridoma Sequencing

Antibody Humanization

Affinity Maturation

VHH Antibody Discovery

Single B Cell Antibody Discovery

Commercial License for CHO-K1

Stable Cell Line Generation

B  CATALOG PRODUCTS

Target Positive Antibody

Isotype Negative Antibody
HIGH THROUGHPUT ANTIBODY/PROTEIN EXPRESSION

Biointron's high-throughput antibody production provides the most cost-effective option for recombinant antibodies, where you may choose to deliver either supernatant or purified antibodies (ug to gram level). From gene synthesis to purified antibodies (IgG, scFv, VHH, bispecific antibodies, etc.), it takes as fast as 2 weeks. Any isotype, format, or antibody fragment can be expressed with no amount limitations.

FEATURES

<table>
<thead>
<tr>
<th>High Throughput</th>
<th>2 Weeks</th>
<th>Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000+ mAb/batch</td>
<td>As fast as 2 weeks for production, including gene synthesis</td>
<td>10+ years of experience on antibody production</td>
</tr>
</tbody>
</table>

QC Standards: SDS-PAGE >95%, Endotoxin <1 EU/mg, SEC-HPLC Detection

WORKFLOW

1. Sequence Design and Synthesis
2. Plasmid Construction and Preparation
3. Transient Expression
4. Delivery
5. Stringent Quality Control
6. Affinity Purification
BISPECIFIC ANTIBODY (BSAB) PRODUCTION

Bispecific antibodies represent a key component of the next-generation of antibody therapy. Bispecific antibodies can target more than two different antigens at the same time. For instance, they can simultaneously bind tumor cell receptors while recruiting cytotoxic immune cells.

Biointron has expressed thousands of bispecific antibodies, and we have extensive experience in bispecific antibody production. Any format can be expressed.

FEATURES

- **High Throughput**
  - 500+ molecules/batch

- **Customized Service**
  - Any format can be produced

- **Fast Turnaround Time**
  - As fast as 3 weeks

CASE STUDY

IgG-scFv

Lane R: Reducing, Lane N-R: Non-Reducing, Lane M: Protein Marker
LARGE SCALE PRODUCTION

Manufacturing therapeutic antibodies or proteins in large quantities is required for many applications, including in vitro studies, in vivo animal studies, and pre-clinical trials. Biointron’s large-scale manufacturing services offers scaled-up production of recombinant antibodies/proteins from milligram to gram levels within weeks.

FEATURES

Consistency
All products come from one batch, SDS-PAGE >95%, SEC-HPLC >95%, endotoxin level <0.1 EU/mg

Fast Turnaround Time
As fast as 3 weeks for gram-level production

High Capacity
Up to the hundred-gram level

CASE STUDY

1000 mg recombinant mAb production in CHO cells

<table>
<thead>
<tr>
<th>Expression results</th>
<th>Yield (mg/L)</th>
<th>SDS-PAGE</th>
<th>SEC-HPLC</th>
<th>Endotoxin (EU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>352</td>
<td>&gt;98%</td>
<td>98.89%</td>
<td>&lt;0.1 EU</td>
</tr>
</tbody>
</table>

Lane M: Marker
Lane 1: Non-Reducing
Lane 2: Reducing

SEC-HPLC Result
HYBRIDOMA SEQUENCING

The antibody sequence is crucial for patent applications (distinguish your antibody from others), recombinant antibody expression, antibody engineering (humanization & affinity maturation), and more. Biointron offers a high-quality hybridoma sequencing service, for the sequencing of variable regions of monoclonal antibodies produced from hybridoma cell lines. Once the sequences are confirmed, the recombinant antibody can be expressed and purified in as fast as 2 weeks.

FEATURES

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' RACE</td>
<td>Simple and reliable</td>
</tr>
<tr>
<td>Fast Turnaround Time</td>
<td>1 week</td>
</tr>
<tr>
<td>100% Accuracy</td>
<td>Cross-verified with 5 independent clones</td>
</tr>
</tbody>
</table>

WORKFLOW

1. Hybridoma
2. mRNA Extraction
3. Reverse Transcription
4. PCR Amplification
5. Antibody Expression (Optional)
6. Bioinformatics Analysis
7. Sequencing (at least 5 positive clones)
8. Subcloning
Recombinant antibody technologies are rapidly becoming available and showing considerable clinical success. However, murine monoclonal antibodies may induce immunogenic responses, potentially limiting their application for humans. Humanized antibodies can overcome these problems and are considered to be a promising alternative therapeutic agent.

Biointron’s antibody humanization service is based on CDR grafting and back mutation with PTM analysis.

**FEATURES**

- **Affinity Guaranteed**
  - No less than 3 times
- **Fast Turnaround Time**
  - As fast as 3 weeks
- **Expertise**
  - 10+ years in humanized antibody design

**CASE STUDY**

- **Mouse IgG**
- **Human IgG**
- **Antibody A**
- **Humanized Antibody A**
AFFINITY MATURATION

**FEATURES**

**FC-MEST™**
Full Coverage Mammalian Expression System

**No-bias Site Saturation Mutagenesis in CDR Region**

**At least 5-fold Affinity Improvement Guaranteed**

FC-MEST™ (Full Coverage Mammalian Expression System) is Biointron’s proprietary platform for affinity maturation. Each amino acid in the CDR region will be mutated to the other 18 amino acids (except Cysteine) with equal ratios. The clones will be expressed in a mammalian system and an ELISA binding assay will be conducted as an initial test, followed by SPR or FACS for further affinity measurement.

**WORKFLOW**

1. Site-directed saturated mutation
2. High-throughput mammalian cell expression
3. ELISA and sequencing to identify hot spots
4. Combinatorial mutation design and characterization

**CASE STUDY**

Mouse chimeric antibody | Humanized antibody | variant 1 | variant 2 | variant 3

Affinity increases 120-fold, 48-fold and 46-fold separately.
VHH ANTIBODY DISCOVERY

SdAb or VHH is a promising next-generation therapeutic antibody technology for cancer immunotherapy and other applications.

Biointron is a well-recognized leader in the field of single domain antibody discovery. Based on our advanced phage display technology and high-throughput antibody expression platform, our scientists are specialized in the production and discovery of specific single domain antibodies.

FEATURES

- Self-owned Alpaca Breeding Base
- Guaranteed 20+ Unique Binders
- High Diversity & Large Capacity

WORKFLOW

1. Antigen Preparation
2. Alpaca Immunization
3. VHH Screening
4. Plasma B Cell Sorting
5. mRNA Extraction & RT-PCR Amplification
6. Phage Display
7. mRNA Extraction & Reverse Transcription
8. Phage Library Generation
9. Library Screening & Biopanning
10. Single Sequence High-throughput Expression Screening
11. Bioinformatics Analysis
12. Basic Detection (Supernatant) Protein Binding
13. Cell Based Binding
14. Sequencing of Positive Clones
15. VHH Expression and Purification
Biointron offers a high-throughput, fully integrated platform for accelerating antibody discovery. Biointron’s high-throughput single B cell screening platform can screen $2 \times 10^6$ plasma B-cells from immunized animals. Antigen specific antibody-secreting cells can be isolated. It allows the detection of potentially rare antibodies or drugs with unique properties.

### ABOUT 2 MONTHS: FROM TARGET TO ANTIBODY SEQUENCE DISCOVERY

**IMMUNIZATION**
- 5-10 BALB/c mouse
- DNA, Protein, Cells
- 1-2 months to maintain repertoire diversity

**PLASMA B CELLS ISOLATION**
- Isolate plasma B cells from spleen and bone marrow

**SINGLE B CELL SCREENING**
- Up to 2E6 plasma B cells are screened
- Protein Binding
- Cell-based Binding
- Sorting positive microdroplets

**HIGH-THROUGHPUT EXPRESSION and VALIDATION**

**SINGLE-CELL SEQUENCING**

### FEATURES

- Antibodies Directly from Primary Plasma Cells
- Time Saving (at least 2-3 months saved)
- High Efficiency (especially for challenging targets, e.g. GPCRs/Ion Channels, etc.)
- More Chances to Identify Rare Antibodies
COMMERCIAL LICENSE FOR CHO-K1

Biointron’s CHO-K1BN cell line, licensed from ECACC (CHO-K1), is suspension-adapted in basic CD media. The cell line can be sublicensed for commercial use, and it meets the requirements for registration in both China and US.

FEATURES

- Worldwide Sublicense
- One-time Sublicense For Multiple Products
- Perpetual & Irrevocable
- Meets the requirements for registration in both China and US

FEATURES OF CHO-K1BN

- Suspension-adapted in basic CD medium and can apply to various medium
- High stability and productivity (> 10 g/L)
- Suitable for monoclonal antibody, bispecific antibody, factors, etc

Adaption of CHO-K1BN

Thawed CHOK1 cell line from ECACC → Culture the adherent CHOK1 cell line → Suspended adaption of CHOK1 cell line by CD serum-free medium to get CHO-K1BN cell line

FOR RECOMBINANT PROTEINS: STABILITY TEST FOR OVER 2 MONTHS

The doubling time of the cells is 22±1 hours. During the plateau period of feed batch, the cell density is 20-25*10^6 cell/ml, making it easier for scale up production. Excellent batch difference, the titer variation is just <10%
STABLE CELL LINE GENERATION

Biointron provides a stable cell line development service for bioproduction purposes. Our stable cell line service, based on ECACC licensed CHO-K1 cells, aims to provide a high-yield guaranteed and speedy timeline to meet your downstream applications.

FEATURES

- **High-yield**
  - >10 gram/L for antibody

- **Expertise**
  - 10+ years of experience, 100+ cell lines delivered

- **One-time Sublicense**
  - For multiple products

WORKFLOW

1. Gene Synthesis and Expression Vector Construction
2. Transfection and Stable Pool Development
3. Single Cell Clone Screening
4. Stability Test for PCB
5. PCB Establishment
6. Single Clone Selection
TARGET POSITIVE ANTIBODY

- To benchmark novel biotherapeutics (biologics) for pre-clinical lead identification.
- For the development of potency assays.

Our research grade biosimilars can be used as positive controls FOR RESEARCH USE ONLY.

SPECIFICATIONS

- Endotoxin < 1.0 EU/mg determined by the LAL method
- Purity > 95% determined by SEC-HPLC

SDS-PAGE Image of Human PDL1/His Protein

SDS-PAGE Image of Anti-PDL1 [Atezolimumab]

The purity was more than 99% as determined by SEC-HPLC

Human PD-L1 Protein, His Tag (Cat. No. H-PDL1-1) can bind Research Grade Biosimilar Anti-PDL1 Antibody [Atezolimumab]

Kinetics analysis for PDL1(Cat: H-PDL1-1) binding to Research Grade Biosimilar Anti-PDL1 Antibody [Atezolimumab](Cat: B2016)(Top dose 40nM, 3-fold dilution)

Observations:
Close KD values were obtained for PDL1 binding to anti-PDL1 antibody compared with historical data (0.4m).
ISOTYPE NEGATIVE ANTIBODY

Choosing the correct isotype control antibody is an important part of most antibody-based experiments. Isotype control antibodies have no relevant specificity, and they enable the researcher to distinguish non-specific backgrounds binding from antigen-specific antibody binding.

Our isotype controls are specific anti-HEL (Hen Egg Lysozyme) and has no known cross-reactivities to humans.

<table>
<thead>
<tr>
<th>Number</th>
<th>Product Name</th>
<th>Number</th>
<th>Product Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>B118301</td>
<td>anti-HEL- Mouse IgG1</td>
<td>B109801</td>
<td>anti-HEL-Human IgG1 (N297A)</td>
</tr>
<tr>
<td>B115101</td>
<td>anti-HEL- Mouse IgG2A</td>
<td>B107803</td>
<td>anti-HEL-Human IgG2</td>
</tr>
<tr>
<td>B192301</td>
<td>anti-HEL- Mouse IgG2A LALA</td>
<td>B109803</td>
<td>anti-HEL-Human IgG3</td>
</tr>
<tr>
<td>B117901</td>
<td>anti-HEL-Human IgG1</td>
<td>B109805</td>
<td>anti-HEL-Human IgG4 (S228P L235E)</td>
</tr>
<tr>
<td>B109802</td>
<td>anti-HEL-Human IgG1 LALA</td>
<td>B107804</td>
<td>anti-HEL-Human IgG4 (S228P)</td>
</tr>
</tbody>
</table>

* FOR MORE PRODUCTS AND RELATED DETAILS, PLEASE CONTACT US AT WWW.BIOINTRON.COM

SDS-PAGE Image of Human IgG1 Isotype Control mAb

The purity was more than 98% as determined by SEC-HPLC

No cross-reactivity (e.g. A549 cells, FACS)

Specific binding to HEL (ELISA)

Body Weight Changes of Mice (BALB/c nude)

HepG2 Human Liver Carcinoma Xenograft Model (BALB/c nude)
• Founded in 2012 in Taizhou, China
• Little Giant of Science and Technology nurturing enterprise
• Chinese National High-tech Enterprise
• ISO-9001:2015 certificate

CONTACT US

www.biointron.com
info@biointron.com
+1(732) 790-8340

Biointron Biological Inc
G122 building, China Medical City, Taizhou, Jiangsu, China

Shanghai R&D/Production Center
Building 1, No. 1-9 Lane 99, Shenmei Road Zhoupu Town, Pudong New District

Biointron Biological USA Inc
20 Bridge St, Bldg B, Metuchen, NJ 08840