IBA BIOLOGICS

www.iba-bioTAGnology.com

Custom Services

Just relax - and leave your lab work to us!

IBA BioTAGnology

IBA Gene- and Protein TAGnologies –
Custom Services provided by IBA Biologics

IBA headquarters, Göttingen

Two specialists, one perfect match:

IBA BioTAGnology – the tool provider & IBA Biologics – your custom service partner!

Being one of the foremost providers of TAG-derived products, the „TAG-company" IBA GmbH is covering the entire production process of recombinant proteins from cloning, transfection, protein expression and purification to detection, assay and immobilization. This wide-range product portfolio is supplemented by a comprehensive custom service offered by IBA Biologics, IBA´s 100 % daughter since April 2007. Using the expertise and access to innovative and proprietary IBA TAGnologies, IBA Biologics accompanies its clients in pharma and biotech industries as a competent partner from early discovery to cGMP production of proteins.

In January 2008, IBA Biologics joined IBA GmbH in the Göttingen headquarters.

Contact our experts at service@iba-go.com for your specific quote.

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Source it out!

For large-scale production of recombinant proteins (or nucleic acids of biological origin) under non-cGMP or cGMP see page 8

Cloning, Protein Expression & Purification Services

Reliable outsourcing from cloning to protein expression and purification!

Our strengths

- Strong expertise in cloning and large scale protein expression
- Application of innovative and proprietary TAGnologies (e.g. StarGate®, *Strep*-tag®)
- Large variety of different vector elements to cover customer needs
- High quality output within short time periods

Outsourcing - fast and cost-effective

Proteomics has generated the demand to work with hundreds of proteins. Due to limited resources and the complexity of technology outsourcing of expression cloning and protein purification may be mandatory to cope with the needs of the post-genomic era.

Quality service and communication

IBA / IBA Biologics are competent research partners and one of the foremost providers of expression cloning and protein purification technologies and products. We are committed to providing flexible, timely, and high quality services. Our contract services can be customized according to your needs.

Typically, we complete a project from subcloning to delivery of a test-scale expression of purified protein within 1-2 months.

Communication with our clients is essential to us. Therefore, we take a collaborative approach in establishing effective working relationships with key client representatives.

Requirements for IBA Biologics' custom services

The customer is requested to provide the complete gene sequence of interest in electronically readable form or its "Genbank" accession number. Any information concerning the biochemistry of the protein of interest would be helpful for planning a suitable expression strategy.

TAGnologies, products and services for recombinant protein production: www.iba-bioTAGnology.com

StarGate® Combinatorial Cloning

These services comprise the precise cloning of the desired gene into IBA's proprietary StarGate® Entry Vectors and subsequently the transfer into the StarGate® Acceptor Vectors with optimal control of expression in *E. coli*, yeast, insect or mammalian cells.

General procedure

- Consulting and planning of the cloning strategy
- Primer synthesis and PCR with proof-reading DNA polymerase (if necessary)
- Alternatively, *de novo* synthesis of the desired gene
- Entry Cloning into the Entry Vector (basis for all further transfer reactions)
- Transfer Cloning into customer-defined Acceptor Vector(s)
- Control sequencing of insert

Entry Cloning (Generation of Donor Vector)

Depending on the gene template provided by the customer, two different Entry Cloning procedures can be performed:

1. Entry Cloning using gene from gene synthesis

The desired gene is chemically synthesized and cloned into the ENTRY Vector. Codon optimization is performed upon request.

2. Entry Cloning using PCR from plasmid DNA

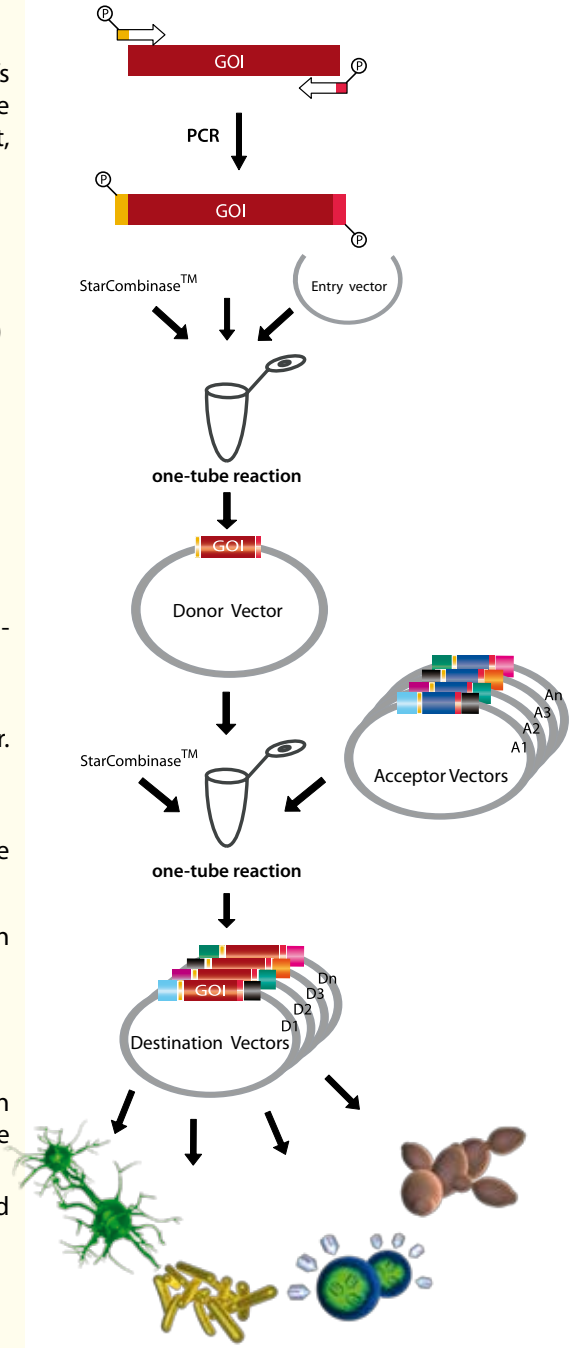
The gene of interest (GOI) is amplified by PCR from a plasmid DNA template and is inserted precisely into the ENTRY Vector.

Additional costs for sequencing will be charged per base pair depending on the gene length.

Time required: 4-8 weeks (depending on the cloning procedure)

Cloning is planned in close cooperation with the client. For example, we can introduce specific point mutations (such as additional restriction sites) into the termini of a gene via PCR cloning.

The original control sequencing print-outs of the gene insert can be provided on request. IBA does not guarantee that the encoded gene will be expressed.



StarGate® - combinatorial cloning in one tube

Transfer Cloning into Acceptor Vectors (Generation of Destination Vectors)

After sequence confirmation, the resulting Donor Vector is the origin for highly parallel subcloning of your gene of interest into a multitude of Acceptor Vectors, each providing different functional elements like host specific promoters and a variety of purification tags. Subsequently, the resulting Destination Vectors are transformed into the corresponding host cells for protein expression.

Please download the up-to-date list of StarGate® Acceptor Vectors from our website: www.stargate-cloning.com

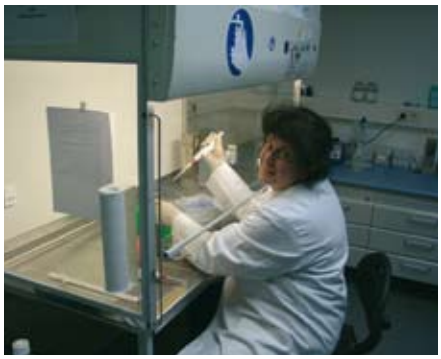
Tailor-made Acceptor Vectors are available on request: service@iba-go.com

For **adaptation of your vectors into StarGate® Acceptor Vectors** please contact our experts at **service@iba-go.com**. We are looking forward to discussing your project.

Cloning into IBA´s Classic Vectors

Standard cloning and expression is based on the StarGate® system. IBA´s classic cloning vectors are used on request.

General Expression Procedure



Custom service in IBA Biologics' laboratories

For all expression systems, i.e. *E.coli*, yeast, insect cells (baculovirus) and mammalian cells, a test phase preceeds the production phase. In the **Test Phase**, expression is performed under standard conditions to evaluate the productivity of the expression system and to determine the culture volume necessary for the production of the amount of protein required by the customer. In the **Production Phase**, expression is performed in agreed culture volumes according to the yield of the recombinant protein as evaluated in the test phase. For details please refer toTable 1.

Table 1: General Expression Procedure

	E. coli	Yeast	Insect (baculovirus)	Mammalia
Test Phase: Expression under standard conditions to determine the culture volume necessary for the production of the required amount of protein.				
Culture volume for test scale expression	Transformation 200 ml	Transformation PCR check 30 ml	Generation of baculovirus 2 ml	Transfection Selection 30 ml
Test affinity purification* and detection	Test affinity purification, SDS PAGE and Western blot	Test affinity purification, SDS PAGE and Western blot	SDS PAGE and Western blot	Test affinity purification, SDS PAGE and Western blot
Production Phase: Expression under standard conditions in culture volumes fixed according to the customer´s requirements. Purification with *Strep*- or 6xHistidine-tag, other tags on request.				
Expression and purification scales	1 L 2 L 5 L 10 L up to 300 L	100 ml 500 ml 1000 ml 5000 ml	High titer 1.0 L 5.0 L 12.5 L	1 L eq. 0.25 m^{2**} 5 L eq. 1.00 m^{2**} ** adherent cells
Affinity purification*	with *Strep*- or 6xHistidine-tag	with *Strep*- or 6xHistidine-tag	with *Strep*- or 6xHistidine-tag	with *Strep*- or 6xHistidine-tag

* Please note that standard affinity purification is performed with *Strep*®-tag and/or 6xHistidine-tag. It can be performed, however, also with other tags, as required by the customer, or without tags. Please get in touch with us to discuss your specific set-up.

Expression in *E. coli* and Purification

with *Strep*-tag® and/or 6xHistidine-tag

Protein purification using other tags on request: service@iba-go.com

General information

Escherichia coli (*E. coli*) is one of the most widely used hosts for the production of heterologous proteins and genetics are far better characterized than those of any other microorganism. A couple of strains with different genotypes are available for different, specific purposes, one of the work horses for recombinant protein expression being *E. coli* BL21.

Recent progress in the fundamental understanding of transcription, translation, and protein folding in *E. coli*, together with the availability of improved genetic tools like the IBA StarGate® technology are making this bacterium more valuable than ever for the expression of complex eukaryotic proteins when protein modifications like glycosylation are not a task.

Test Phase (please refer to Table 1)

- *E. coli* test culture (200 ml) and induction of protein expression
- *Strep*-Tactin® and/or Ni-NTA affinity purification, SDS-PAGE, Western blot
- Estimation of the culture volume required to produce the requested amount of protein
- Time required: 1-2 weeks

Production Phase (please refer to Table 1)

- *E. coli* culture in the appropriate volume, preparation of protein extract and *Strep*-Tactin® and/or Ni-NTA affinity purification
- Optimization

A set of different *E. coli* test cultures (200 ml) under various expression conditions and/or different affinity purification attempts can be performed. This set of experiments will be planned in close consultation with the client and depends on the initial results of the *E. coli* test scale expression.

Expression in *S. cerevisiae* and Purification

with *Strep*-tag® and/or 6xHistidine-tag

Protein purification using other tags on request: service@iba-go.com

General information

The yeast *Saccharomyces cerevisiae* is an eukaryotic host, which produces soluble recombinant proteins and is capable of introducing post-translational modifications into the protein. The expression is performed in a protease deficient strain. The expression vector for *S. cerevisiae* is based on a multi-copy plasmid containing a strong and rapidly induced promoter.

Test Phase (please refer to Table 1)

- Transformation of the expression vector containing the target DNA into yeast and generation of a recombinant *S. cerevisiae* clone
- Screening for positive clones via PCR, selection of an appropriate clone
- Test expression of 30 ml culture volume
- *Strep*-Tactin® and/or Ni-NTA affinity purification, SDS PAGE, Western blot
- Estimation of the required culture volume to obtain the requested amount of protein
- Time required: 3-4 weeks

Production Phase (please refer to Table 1)

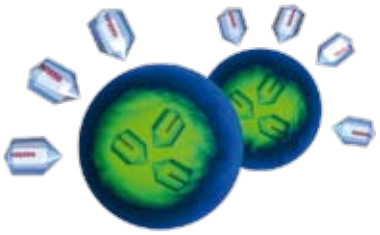
- Culture of *S. cerevisiae* in the appropriate scale and induction of protein expression
- Cell harvest and lysate preparation
- *Strep*-Tactin® and/or Ni-NTA affinity purification

Protein purification using other tags on request: service@iba-go.com

Expression in Insect Cells (baculovirus) and Purification

with *Strep*-tag® and/or 6xHistidine-tag

General information



The Baculovirus insect cell expression system is widely used for the production of precisely matured, folded and processed recombinant proteins. In contrast to a prokaryotic expression system like *E. coli*, insect cells are able to glycosylate the proteins although the glycosylation pattern is not identical to mammalian cells. This unique tool usually yields high amounts of the produced protein, making it highly cost effective in comparison to other eukaryotic expression systems.

Test Phase (please refer to Table 1)

- Cotransfection of insect cells with transfer vector DNA and linear virus DNA, generation of recombinant viruses, plaque purification
- Test expression of 6 recombinant viruses
- Western blot analysis
- Estimation of the required culture volume to obtain the requested amount of protein
- After having performed the test expression the customer receives supernatants (2 ml) or cell pellets of 6 test expression cultures for his own tests
- Time required: 4-8 weeks

Production Phase (please refer to Table 1)

- High titer virus stock production
  In case of detectable and functional recombinant protein in at least one of the 6 samples, the recombinant baculovirus has to be amplified to get a high titer virus stock (100 ml of 5 x 10^7 to 1 x 10^8 pfu/ml)
- Time required: 1-3 weeks
- Culture in the appropriate scale and protein expression
- Cell harvest and lysate preparation
- Ni-NTA affinity purification

Expression in Mammalian Cells and Purification

with *Strep*-tag® and/or 6xHistidine-tag

Protein purification using other tags on request: service@iba-go.com

General information

Mammalian cell lines (BHK, CHO, HEK 293 etc.) are widely used for the production of recombinant glycoproteins, e.g. vaccines, enzymes as well as hormones and immunobiologicals (antibodies, interleukins, lymphokines).

Test Phase (please refer to Table 1)

- Transfection of mammalian cells
- Transient protein production for fast generation of small protein amounts
- Cell harvest and lysate preparation or supernatant
- *Strep*-Tactin® and/or Ni-NTA affinity purification, SDS-PAGE, Western blot
- Estimation of the required culture volume to obtain the requested amount of protein
- Time required: approximately 2 weeks

Process development on request

- Adaption to serum-/protein-free media for the production of biopharmaceuticals
- Optimization of freezing conditions in serum-free media
- Optimization of cultivation conditions: CO_2 supply, glutamine/ GlutaMAX media
- Analytics as required by the customer: e.g. cell number and viability, glucose/lactate, amino acid analysis, pH measurement, ammonium determination

Production Phase (please refer to Table 1)

- Transient transfection of mammalian cells is a powerful technology for the fast production of small amounts of recombinant proteins. Depending on adherent or suspension cells the customer can choose between 0,25 m^2 to 1 m^2 corresponding to 1L to 5L cultures.
- *Strep*-Tactin® and/or Ni-NTA affinity purification

GMP Contract Manufacturing: your gateway to clinical trials

Cell banking – Process development (non-cGMP to cGMP) – Production for clinical trials

To discuss your project please contact bertram@iba-go.com

cGMP: Current Good Manufacturing Practice is a set of rules to ensure identity, potency, safety and purity of the product.

cGMP processes are carried out in respective clean rooms

IBA Biologics offers a variety of custom services (non-cGMP or cGMP on demand)

Consulting

Based on our expertise and knowledge in process development we are ready to assist you with an on-site analysis and evaluation of your project.

Construction of stable recombinant strains and cell lines

Strains and cell lines for the production of biologically active recombinant proteins using IBA's world-wide patented *Strep*-tag® technology.

Vector construction

Vector construction for an efficient delivery of the gene of interest in a given cell line according to customer-specified needs.

Cell banking

Manufacture and storage of master cell banks (MCB), working cell banks (WCB) and virus stocks.

Quality control

Development and validation of analytical procedures for the determination of identity, purity, safety, potency and content for in-process control and lot release testing.

Production of pre-clinical material for toxicological investigations

Process design and optimization for a rapid change-over to a production for clinical phases.

Production of clinical material for all phases of clinical trials

Complete process validation; production of bulk active pharmaceutical ingredients (API).

Microbial fermentation and cell culture

Production of non-cGMP-grade and cGMP-grade bulk material in BL1 and BL2 culture adaptation to serum-free or protein-free media; process development; optimization and scale-up; process validation; cultivation and primary separation options for microbial processes from laboratory scale up to production scale; cultivation in various bioreactor geometries.

Downstream processing (DSP)

Primary separation, purification and polishing of proteins and nucleic acids

The comprehensive range of downstream processing services we offer include laboratory, pilot and production scale: cell harvesting and disruption; recovery and solubilization of inclusion bodies; microfiltration and ultrafiltration; chromatography (size exclusion, ion exchange, affinity); formulation, freeze drying and lyophylization.

Custom Monoclonal Antibody Service

Mouse and Rat

NEW: mAB from Rat!

A fast and flexible service according to your needs

Protein analysis cannot be imagined today without monoclonal antibodies which are mainly produced by mice. The use of mice as immunized animals, however, is posed to be a challenge in case the antibodies are intended to be raised against mouse proteins. Moreover, for some assays the use of antibodies of different species is essential. Therefore, we are now also offering rat antibodies using the well-established mouse antibody service.

Requirements and procedure

Phase I / Immunization

For immunization we require 1 mg of purified protein* or 3 mg of purified peptide. As a general procedure we immunize three Balb/c mice or two Lou/c rats with 90 to 150 µg peptide each. If required, we can switch to other mouse strains in the case the provided antigen is critical concerning its immunogenicity (e.g. mouse proteins or peptides).

Mice and rats are immunized over a period of 17 days every second day and one day before fusion. Depending on the antigen we can use different immune stimulating agents like Gerbu or incomplete Freund's Adjuvant combined with CpG oligo-nucleotides (A. M. Krieg, 2002; Annu. Rev. Immunol. 20: 709-760).

* if a Western blot screening is requested (cat. no. 2-2601-004), one additional mg protein is required.

Phase II / Fusion

After immunization, cells from knee lymph knots of all three mice or of two rats are prepared and fused with Ag8 myeloma cells in the presence of PEG 4000.

Phase III / Selection, ELISA screening

After completion of cell fusion the cells are plated on 24-well plates which results in 360 oligo clones each usually consisting of 5 - 10 clones. Cells are cultivated for 10 days in selective and conditioned Optimem medium selecting for fused cells only.

Within the next two days, the clones of interest have to be selected. All 360 oligo clone supernatants are screened by ELISA for antibody positive wells.

Phase IV / Western blot or immunofluorescence screening (optional)

For the next screening step we offer three options:

a) A maximum of 72 oligo clone supernatants are tested in Western blots.

b) A maximum of 360 oligo clone supernatants are screened by immunofluorescence against target cells provided by the customer (e.g. cells transfected with fluorescently labeled fusion protein).

c) A maximum of 20 oligo clone supernatants (5-10 ml) are shipped to the customer for screening under his own conditions.

Phase V /Subcloning and production

Usually screening is completed within a period of three weeks. The customer selects one (or more) oligo clone(s) which is (are) subsequently subcloned to monoclonality by the limiting dilution method. Isolated colonies are tested by ELISA, and positive ones are selected to be expanded for the next cycle of cloning. Several cycles of cloning may be required in order to obtain a true, stable clone, which is subsequently used to produce 100 ml supernatant of the monoclonal antibody. Cells originating from the positive clones are frozen as back up. As part of our service, we are storing the clones until the next production.

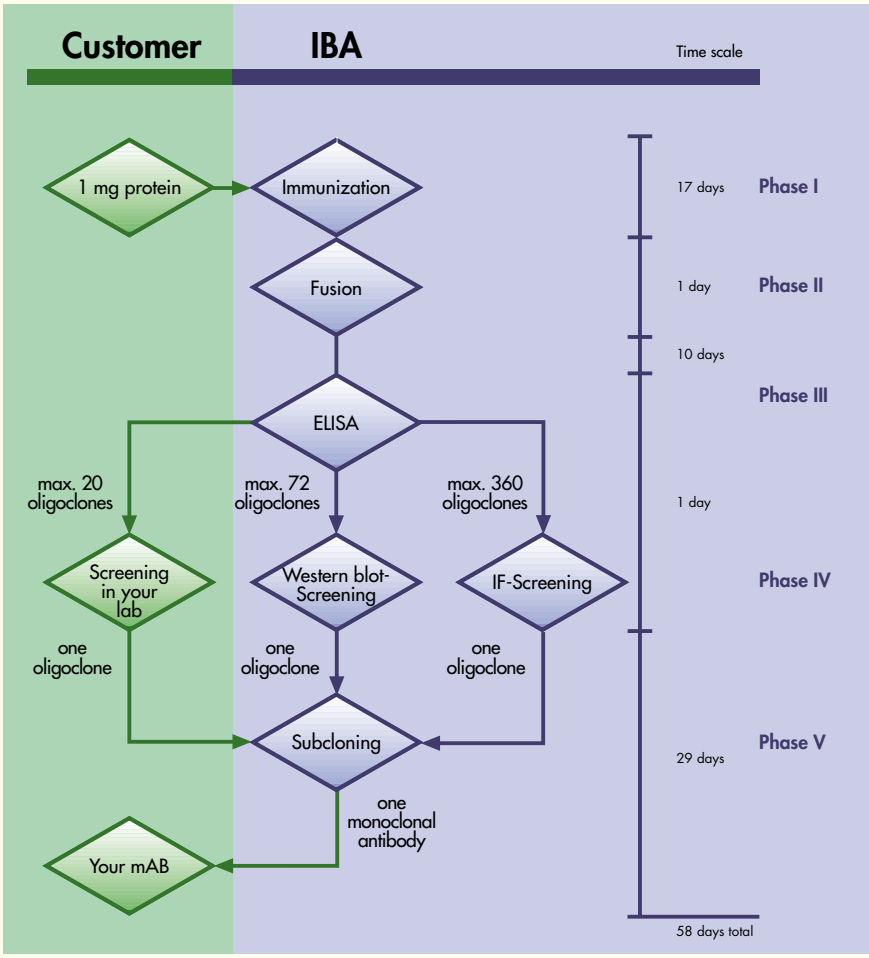
Contact our experts at service@iba-go.com to discuss your project!

Antibody production

Different production scales are available starting at 100 ml supernatant up to 100 mg purified antibody or more.

We also offer antibody production services from hybridoma cell lines provided by the customer. Please contact us for further information.

We appreciate if you have any comments, questions or suggestions. Please get in touch with us at service@iba-go.com.



Flow-chart of antibody production

Standard protocol of mAB generation from mice or rats	
Phase I/Immunization	3 Balb/c **mice** or 2 Lou/c **rats**, 17 days immunization, pre-bleeding and immune serum on request
Phase II/Fusion	Preparation of lymphocytes, fusion with myeloma cell line
Phase III/Selection	Selection, ELISA screening
Phase IV/optional Western blot screening	Screening of max. 72 ELISA positive oligoclones in a Western blot against the antigen provided by the customer
Phase IV/optional IF	Screening of max. 360 oligoclones in an immunofluorescence assay against target cells provided by the customer
Phase V/Subcloning	Subcloning by limiting dilution, second ELISA screening, production of 100 ml supernatant of selected antibody

Framework Agreement and Licensing

General remarks about IBA's / IBA Biologics' protein expression and purification services

- If for any reason a successful PCR product cannot be obtained from a template supplied by the customer and/or the sequence information, a basic fee will be charged for our cloning attempts only.
- In case, the *Strep*- or 6xHistidine tag was used, the purified protein is supplied as eluted from the *Strep*-Tactin® or Ni-NTA affinity column in elution buffer containing desthiobiotin or imidazole, respectively.
- Protein concentrations are determined using the theoretical extinction coefficient at 280 nm of the recombinant protein, which is determined by the addition of the extinction coefficients of the tryptophane and tyrosine residues present in the recombinant protein or by a colorimetric assay according to Bradford, with BSA as reference protein.
- Original print-outs of the control sequencing are available on request.

Integrity, secrecy and licensing

Our custom service requires the exchange of sensitive data. Therefore, all proprietary information and intellectual property transferred to IBA/IBA Biologics is kept absolutely confidential and is covered by a secrecy agreement if requested.

As a service provider we do not intend to claim any rights on proteins which we manufacture for our clients as long as the proteins are used for in-house research purposes only. A separate license for use of the *Strep*-tag® technology is necessary, however, if direct commercialization of ordered proteins is intended. This is also the case if clients use the *Strep*-tag® technology for the production of recombinant proteins in their own facilities with subsequent commercialization. IBA is authorized and ready to grant licenses for those purposes.

Information about licenses for commercial use of 6xHistidine-tag proteins is available from QIAGEN GmbH, Qiagen Strasse 1, D-40724 Hilden, Germany.

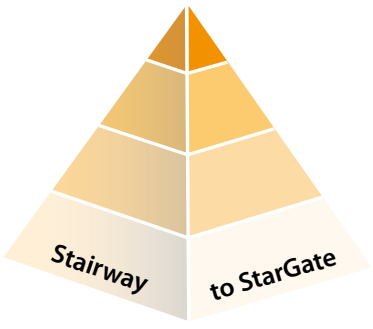
IBA is not obliged to accept orders. Shipping costs are charged separately.

IBA patents, licensing and trademarks

Strep-tag® technology for protein purification and detection is covered by world-wide patents (US5506121; DE4237113; JP3865792; UK2272698; FR9313066); the tetracycline promoter based prokaryotic expression system is covered by US5849576 and EP759997; *Strep*-Tactin® is covered by US6103493 and patent applications in Europe; StarGate® cloning, *Strep*tamer® technology for T-cell purification and One-STrEP-tag technology are protected by world-wide patent applications. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Cloning, expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use.

For details regarding our open access policy for the use of StarGate® please get in touch with us.

Strep-tag®, *Strep*-Tactin®, *Strep*tamer® and StarGate® are registered trademarks of IBA GmbH.



StarGate®

NEW!

The new dimension of combinatorial cloning

The comprehensive StarGate® product portfolio such as Acceptor Vectors and Cloning Kits enables the systematic screen for optimal protein expression.

www.stargate-cloning.com

- Easy-to-handle sub-cloning procedure
- Minimal modification of the gene of interest (≤ 2 aa)
- Systematic screening of different tags and promoters
- High efficiency cloning due to directed reaction
- High protein expression due to optimal host selection
- Mutagenesis kit available
- Multiple gene fusions possible

StarPrimer D'Signer Software 2.0

Innovative tool for StarGate® Mutagenesis System

The StarPrimer D'Signer is an easy-to-use software to facilitate the design of primers for introducing mutations into a gene of interest (GOI), which is then transferred into a StarGate Entry Vector to create a Donor Vector.

In addition, the software can also be used for primers designed for Standard StarGate Entry Cloning.

The Microsoft Windows software comes free of charge with the system and is also available for download at http://www.iba-go.com/download.html (<1 MB).

IBA is represented by a world-wide network of distributors

Please select your local distributor from our website:
www.iba-bioTAGnology.com/distributors.html

Request further information as well as the complete IBA Portfolio Folder at www.iba-bioTAGnology.com and subscribe to our e-newsletter!

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