

CUSTOM BACULOVIRUS SERVICES

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.

RELIATech offers a variety of custom services for gene expression (especially signal proteins) in insect cells. This “*Baculovirus Expression Service*” is divided into a series of steps which can be performed either individually or in combination so that you can select the steps you actually need. These services are designed to adequately cover most of your needs, from gene cloning into the Baculovirus transfer vector to recombinant baculovirus construction, high titer recombinant baculovirus production, bulk recombinant protein production as well as protein purification. All steps are made in serum-free medium to ensure safety and to yield a high purity of the recombinant protein. Along with the regular project updates, you will receive progress reports at the end of each step of your program. You will then be asked to authorize us to proceed to the next step. Each step is described hereafter:

A1) Gene cloning within a baculovirus transfer vector

The complexity of this step is highly dependent on the gene to be expressed. Conforming to customer's needs, RELIATech can perform all the experiments, including gene modification, prior to insertion into a transfer vector. Alternatively, assistance is provided to the customer allowing him to choose the right transfer vector and the right conditions to virtually ensure the optimal gene expression.

Material needed

- 10-30 µg purified and characterised plasmid cDNA bearing the gene to be expressed and containing the appropriate restriction sites for subcloning.

The service includes

- Subcloning of the cDNA into the baculovirus transfer vector pBacPAK-8/-9 or in a transfer vector chosen by the customer.

Note: If there are no appropriate restriction sites, the cDNA first has to be subcloned into another expression vector, e.g. pCR2.1, before cloning into the baculovirus transfer vector!

- Plasmid-preparation of 10 recombinant clones.
- Analysis by agarose gel electrophoresis.
- Mid-scale plasmid preparation of a positive clone.

Provided to the customer

- a baculovirus transfer vector containing the cDNA of interest.
- A detailed report sheet.

Expected time range:

1- 4 weeks

A2) Gene cloning within a baculovirus transfer vector including a „tag“

With respect to the purification procedure we would recommend introducing a “tag” at the N- or C-terminal end of the recombinant protein. If the recombinant protein is used mainly in *in vitro* experiments (e.g. cell culture studies) we recommend for example a “His-tag” (6x Histidin) or a “*Strep*-tag II” (8 amino acids) [www.iba-go.de]. The “tag” is normally too small to interfere with the activity of the protein. For “*Strep*-tag II” the possibility exists to create an authentic protein by cleavage of the tag. If the recombinant protein is needed for animal studies (e.g. mouse, rat) we recommend the use of an “Fc-tag” (about 26 kDa) to increase the stability and half-life of the protein in the circulation. As an “Fc-tag” RELIA**Tech** can offer the human IgG1, the mouse IgG 2b or the rat IgG 2a Fc-fragments.

Material needed

- 10-30 µg purified and characterised plasmid cDNA bearing the gene to be expressed.

The service includes

- Generation of specific oligonucleotide primers containing the appropriate restriction sites for subcloning with a “tag”.
- PCR with the customer’s cDNA as template.
- Characterisation by agarose gel electrophoresis.
- Subcloning of the PCR-fragment in the baculovirus transfer vector pBacPAK-8/-9 or in a transfer vector chosen by the customer, containing the “tag” chosen by the customer.
- Plasmid-preparation of 10 recombinant clones.
- Analysis by agarose gel electrophoresis.
- Mid-scale plasmid preparation of a positive clone.
- Verification by sequencing.

Note: All cDNA's generated by PCR have to be completely sequenced due to possible mutations!

Provided to the customer

- a baculovirus transfer vector containing the cDNA of interest.
- A detailed report sheet.

Expected time range:

4- 6 weeks

B) Production of a recombinant baculovirus

Material needed

- 10-30 µg purified and characterised transfer vector cDNA bearing the gene to be expressed. The vector is either provided by the customer or prepared at RELIA*Tech* if service A was also performed.

The service includes

- Ethanol precipitation of transfer vector DNA for sterilisation and quality control by agarose gel electrophoresis.
- Cotransfection of insect cells with transfer vector DNA and linear baculovirus DNA.
- Plaque purification of 6 recombinant viruses from cotransfection supernatant.
- Cell infection of 3×10^6 insect cells with each recombinant and preparation of virus stocks.
- Cell infection of 3×10^6 insect cells with each virus stock for preparation of cell supernatant and cell pellet.

Provided to the customer

- 2 ml of each recombinant virus stock.
- 2 ml of each supernatant to allow the customer to detect the protein of choice.
- or alternative 3×10^6 of each infected cell pellet to allow the customer to detect the protein of choice.
- A detailed report sheet.

Note: On customer request, RELIA*Tech* can determine the protein expression level by Western blotting, if appropriate antibodies are available!

Expected time range:

7- 10 weeks

C) High titer stock production of a recombinant baculovirus

Material needed

- 2 ml of recombinant virus stock, preferably with a known viral titer. The sample is either provided by the customer or prepared at RELIA*Tech* if service B has been performed.

The service includes

- Infection of 0.75×10^8 insect cells and harvesting of supernatant at 72 h post-infection (100 ml virus stock!).

Note: Upon customer request, this service can be performed with or without serum!

- Viral titer determination of the viral stock using plaque assay.

Provided to the customer

- 100 ml of recombinant virus stock at about 5×10^7 to 1×10^8 pfu/ml.
- A detailed report sheet.

Expected time range:

3 – 4 weeks

D) Time course assay of protein production

Material needed

- 10 ml (for study in flasks) or 100 ml (for study in 500 ml spinners) of high titer stock of a recombinant virus. The sample is either provided by the customer or prepared at RELIA*Tech* if service C has been performed.

The service includes

- Infection with the recombinant virus and control virus of 10 flasks or 2 spinners containing insect cells.
- Harvesting of samples (cells and supernatant) of recombinant and control viruses at 24 h, 48 h, 72 h, 96 h and 120 h post-infection.

Note: This step is optional. Normally, the protein yields are best between 72-96 h post-infection!

Provided to the customer

- The harvested samples corresponding to the recombinant virus, to determine the relative amounts of the recombinant proteins at different times post-infection.
- Samples corresponding to the control virus.
- A detailed report sheet.

Note: Upon customer request, RELIA*Tech* can determine the protein expression level by Western blotting, if appropriate antibodies are available!

Expected time range:

2 – 4 weeks

E) Large-scale production of proteins from recombinant baculovirus

Material needed

- 10 ml to 1 L of high titer recombinant virus stock depending on the final production volume needed. The sample is either provided by the customer or prepared at RELIA*Tech* if service C has been performed.

The service includes

- Direct infection of insect cells in spinner flasks at the desired volume.
- Harvesting of the cells and supernatant.

Provided to the customer

- Cell pellet or supernatant corresponding to the infected culture volume.
- A detailed report sheet.

Expected time range:

4 – 6 weeks

F) Protein purification from recombinant baculovirus

The purification of a recombinant protein is the most complex step in the whole cascade. Whereas the cloning of a gene into a transfer vector is often highly dependent on the cDNA fragment size, the purification depends on the biochemical characteristics of the individual protein (e.g. amino acid composition, IP value, glycosylation, stability). In addition, the type of protein (e.g. secreted protein, cytoplasmatic protein, membrane-bound protein, mono- or dimeric protein) also plays a very important role for the purification procedure.

The addition of a “tag” to a protein either at the N- or C-terminal end enables, at the first approach the purification or at least enrichment of the protein using standard purification protocols (e.g. protein A or G Sepharose for the Fc-tag). Furthermore, if there are no specific antibodies available one can use commercially available “tag”-specific antibodies for protein detection as well as for monitoring all the purification steps. Please contact our technical staff for more details.

Note: This part is offered only in combination with step E and a recombinant protein containing a “tag”! For purification of recombinant proteins without a known purification protocol the price depends on the actual time and material needed!

Material needed

- Cell pellet or supernatant from service E.

The service includes

- Preparation of supernatants or cell pellets for purification.
- Preparation of columns and buffers.
- Purification by affinity chromatography.
- Analysis of all fractions by SDS-PAGE and/or Western blotting using a „target-” or „tag”-specific antibody.
- Determination of protein purity by SDS-PAGE and subsequent silver staining.

Provided to the customer

- All recombinant protein purified from the customer requested volume.
- A detailed report sheet.

Expected time range:

Depends on the desired volume!
