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Baculovirus expression vector pAc-λ-CH3

Vector name	pAc-λ-CH3 (for details see page 4)
Category	Baculovirus cassette vector for the expression of human, humanized or chimeric IgG(λ) in insect cells
Application	Cloning of heavy and light chain variable domain genes of scFv antibody fragments selected from hybridomas, single B-lymphocytes or phage display libraries. Cloning sites: *Xho*I and *Nhe*I for heavy chain V_H genes *Sac*I and *Hind*III for light chain V_L(λ) genes. Expression of immunoglobulin heavy and light chains in insect cells and secretion of assembled IgG(λ) antibodies into the supernatant
Vector size	7360 bp
Resistance	The vector contains an Ampicillin resistance marker gene.
Purification	Qiagen Plasmid Purification Kit
Dilution	0.5 µg/µl in TE buffer
Stability/Storage	1 year at -20 °C
Quantity	1 vial
Volume	10 microliter

For research use only !

Reference

1. Liang, M., Dübel, S., Li, D., Queitsch, I., Li, W. and Bautz, E.K.F. (2001) Baculovirus expression cassette vectors for rapid production of complete human IgG from phage display selected antibody fragments. J. Immunol. Meth. 247, 119-30.
2. Liang, M. and Dübel, S. (2001) Expresssion of antibody fragments using the Baculovirus system. In: Antibody Engineering, ed: Kontermann, R. and Dübel, S. Springer Verlag, Heidelberg.

Cat. No. PR3002

Baculovirus Expression Vectors pAc-κ-CH3, pAc-κ-Fc pAc-λ-CH3, pAc-λ-Fc

Baculovirus cassette vectors for the expression of human, humanized or chimeric IgG in insect cells

Cat. No.:	*Vector Name*	*Quantity*
PR3000	**pAc-κ-CH3**	**5.0 µg**
PR3001	**pAc-κ-Fc**	**5.0 µg**
PR3002	**pAc-λ-CH3**	**5.0 µg**
PR3003	**pAc-λ-Fc**	**5.0 µg**
Storage upon receipt:	**-20 °C**	
***In vitro* Test**	**FOR RESEARCH USE ONLY**	

1. Introduction

Within the past decade, recombinant antibody technologies have been widely used to produce various single-chain Fv or Fab antibody fragments of different specificity. The randomized combination of cloned variable heavy and light chain immunoglobulin gene fragments further allowed the construction of human antibody libraries, which enable today the isolation of specific scFv or Fab antibody fragments against particular antigens e.g. by phage display. For many applications, however, it is required to reassemble the variable regions of the selected antibody with immunoglobulin constant regions to generate complete antibody molecules.

The baculovirus expression system has already been established as a reliable system for the production of immunoglobulins (Nesbit *et al*., 1992; Liang *et al*., 1997, 2001). Even the development of cassette vectors for the production of human antibodies have been reported previously (Poul *et al*., 1995a,b). However, these systems are based on a combination of two different vectors which separately served for the expression of light and heavy chains. The required careful and time consuming adjustment of the two respective recombinant baculovirus titers now becomes obsolete, since PROGEN offers new single baculovirus cassette vectors with authentic IgG heavy and light chain signal sequences for the rapid production of complete chimeric, humanized and human IgG antibodies in recombinant baculovirus infected insect cells (Liang *et al*., 2001). The vectors were specifically designed for cloning of heavy and light chain Fv or Fab gene fragments isolated from hybridomas, individual B cell clones as well as antibody libraries.

2. Vector Design

To achieve the expression of complete immunoglobulins, PROGEN offers a set of baculovirus cassette vectors for the convenient insertion of heavy and light chain Fv or Fab domain coding regions. The vectors are based on the backbone of pAcUW51 (Pharmingen), which contains the f1 origin of phage DNA replication, an ampicillin resistance gene for selection in *E. coli*, the two baculovirus expression promotors of polyhedrin and p10 and SV40 transcription terminators. The gene elements for IgG expression were cloned successively into pAcUW51. The heavy chain gene cassette preceded by the authentic IgG signal sequence from IgG1 (subgroup VHIII) is located under control of the polyhedrin promotor. In pAc-κ-CH3 and pAc-λ-CH3 the recognition sites of the restriction endonucleases *Xho*I and *Nhe*I allow the insertion of a VH gene fragment. The entire coding region of the human IgG1 constant domains are located downstream thereof. The vectors pAc-κ-Fc and pAc-λ-Fc were instead designed for the insertion of a Fab fd gene fragment using the recognition sites of the restriction endonucleases *Xho*I and *Spe*I. The light chain gene cassette elements, starting with the authentic signal sequence of the human kappa chain in pAc-κ-CH3 and pAc-κ-Fc or lambda chain in pAc-λ-CH3 and pAc-λ-Fc, were placed in opposite orientation to the heavy chain operon under control of the p10 promotor.

The recognition sites of the restriction endonucleases *Sac*I and *Hind*III allow the insertion of a VL gene fragment into pAc-κ-CH3 and pAc-λ-CH3. The original HindIII site in the pAcUW51 backbone was therefore removed. The coding region of the human constant kappa light chain is located downstream of the cloning sites. The vectors pAc-κ-Fc and pAc-λ-Fc were designed for the insertion of a Fab light chain gene fragment using the recognition sites of the restriction endonucleases *Sac*I and *Eco*RV (Liang *et al*., 2001).

3. Material Required

- Equipment for DNA cloning
- Basic cell culture equipment

- Insect cell line Sf9 (Pharmingen or Invitrogen)
- BaculoGold DNA, Transfection buffer A and B set (Pharmingen)

4. Contents

5.0 µg purified plasmid DNA, 0.5 µg/µl.

5. Preparation of Reagents

The DNA should be diluted and transfered into *E. coli* cells as described in standard protocols. Use an overnight culture of a single clone to extract enough DNA for the following cloning procedures.

6. Cloning procedure

The immunoglobulin heavy and light chain Fv or Fab domain coding regions must be cloned in-frame into the selected baculovirus expression vector (see attached graphic). To clone a VH gene fragment into pAc-κ-CH3 or pAc-λ-CH3, the recognition sites of the restriction endonucleases *Xho*I and *Nhe*I are recommended. The corresponding VL gene fragment should be introduced by using the recognition sites of the enzymes *Sac*I and *Hind*III. To clone a Fab fd gene fragment into pAc-κ-Fc or pAc-λ-Fc, the recognition sites of the restriction endonucleases *Xho*I and *Spe*I are recommended. The corresponding Fab light chain gene fragment including its terminal stop codon should be introduced by using the recognition sites of the enzymes *Sac*I and *Eco*RV. The cloning sites of the selected Fv or Fab gene fragments have to match the corresponding amino acid positions given by the vector, which were calculated from the first start codon of IgG heavy and light chain mRNA coding regions. Fab genes derived from pComb3 phagemid vector systems (Barbas *et al*., 1991) can be directly cloned into pAc-κ-Fc or pAc-λ-Fc. To clone Fv or Fab genes derived from other systems, the selected gene fragments may need to be reamplified by PCR, thereby introducing the restriction endonucleases sites at correct positions (Liang and Dübel., 2001). After cloning, we recommend to check the sequence of the inserts to confirm that no mutation occured in the open reading frames of the completed IgG heavy and light chain genes. The vector is now ready-to-use for the generation of recombinant baculoviruses for IgG expression in infected insect cells.

7. Baculovirus transfection

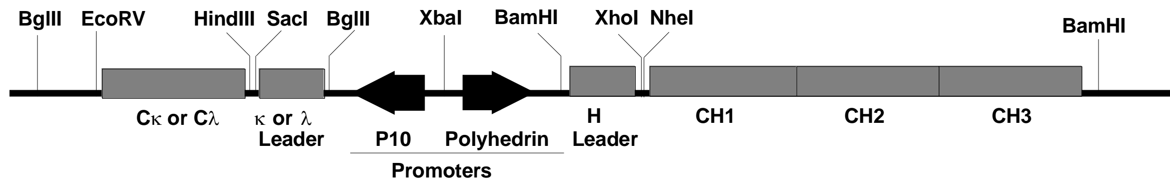
We recommend to use the baculovirus transfection kit from Pharmingen providing lineralized and modified AcNPV baculovirus DNA (BaculoGold DNA). The antibody genes supplied on PROGEN´s transfer vector are flanked by regions homologous to AcNPV. When the lineralized and modified baculovirus DNA is co-transfected with PROGEN´s shuttle vector containing the Fv or Fab domain coding regions into Sf9 insect cells, recombination takes place between the homologous regions, resulting in recombinant baculoviruses carrying and expressing the foreign genes. The baculovirus transfection kit as well as the Sf9 insect cells should be handled as recommended by the manufacturer. The expressed and secreted antibodies from recombinant baculovirus infected insect cells can be purified from the supernatant as described in standard protocols (Liang and Dübel., 2001; Liang *et al*., 2001).

8. Reference

3. Barbas, C.F. 3rd, Kang, A.S., Lerner, R.A. and Benkovic, S.J. (1991) Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. Proc. Natl. Acad. Sci. USA. *88*, 7978-82.
4. Nesbit, M., Fu, Z.F., McDonald-Smith, J., Steplewski, Z. and Curtis, P.J. (1992) Production of a functional monoclonal antibody recognizing human colorectal carcinoma cells from a baculovirus expression system. J. Immunol. Methods *151*, 201-8.
5. Poul, M.A., Cerutti, M., Chaabihi, H., Devauchelle, G., Kaczorek, M. and Lefranc, M.P. (1995a) Design of cassette baculovirus vectors for the production of therapeutic antibodies in insect cells. Immunotechnology *1*, 189-96.
6. Poul, M.A., Cerutti, M., Chaabihi, H., Ticchioni, M., Deramoudt, F.X., Bernard, A., Devauchelle, G., Kaczorek, M. and Lefranc, M.P. (1995b) Cassette baculovirus vectors for the production of chimeric, humanized, or human antibodies in insect cells. Eur. J. Immunol. *25*, 2005-9.
7. Liang, M., Guttieri, M., Lundkvist, A. and Schmaljohn, C. (1997) Baculovirus expression of a human G2 specific, neutralizing IgG monoclonal antibody to Puumala virus. Virology *235*, 252-60.
8. Liang, M. and Dübel, S. (2001) Expression of antibody fragments using the Baculovirus system. In: Antibody Engineering, ed. Kontermann, R. and Dübel, S., Springer Verlag, Heidelberg.
9. Liang, M., Dübel, S., Li, D., Queitsch, I., Li, W. and Bautz, E.K.F. (2001) Baculovirus expression cassette vectors for rapid production of complete human IgG from phage display selected antibody fragments. J. Immunol. Meth. *247*, 119-30.

See page 4 for vector details.

pAc-κ-CH3 and pAc-λ-CH3



VH insertion region of pAc-κ-CH3 and pAc-λ-CH3

```
                                                 XhoI            NheI
ggatccaccatggagttt..42bp..cagtgtgaggtgcagctgctcgagcgtctcctcagctagcaccaagggc..1031bp..tagggatcc
BamHI    M  E  F          Q  C  E  V  Q  L  L  E            A  S  T  K  G            *  BamHI
            H Leader                             aa24            aa1     CH1
```

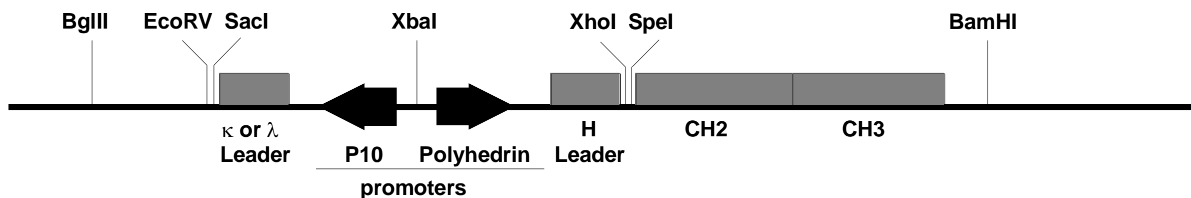
Vκ insertion region of pAc-κ-CH3

```
                                            SacI          HindIII                     EcoRV
agatctcaccatggaaacc..45bp..accggagaaattgagctcaggggaccaagcttgagatc..324bp..tagttctagatatcagatct
BglII     M  E  T          T  G  E  I  E  L          T  K  L  E  I           *             BglII
             Kappa Leader                   aa23          aa5   FR4
```

Vλ insertion region of pAc-λ-CH3

```
                                           SacI           HindIII                     EcoRV
agatctagcatggcctgg..45bp..gcccagtctgtggagctcggagggaccaagcttaccgtc..321bp..tagttctagatatcagatct
BglII    M  A  W          A  Q  S  V  E  L           T  K  L  T  V           *             BglII
            Lambda Leader                  aa23           aa5   FR4
```

pAc-κ-Fc and pAc-λ-Fc



Fd insertion region of pAc-κ-Fc or pAc-λ-Fc

```
                                                 XhoI             SpeI
ggatccaccatggagttt..42bp..cagtgtgaggtgcaactgctcgagcggtgacaaaactagtacatgccca..630bp..tagggatcc
BamHI    M  E  F          Q  C  E  V  Q  L  L  E            T  S  T  C  P            *  BamHI
            H Leader                             aa24             aa106    CH2
```

Kappa chain insertion region of pAc-κ-Fc

```
                                            SacI         EcoRV
agatctcaccatggaaacc..45bp..accggagaaattgagctcacgcaggatatcagatct
BglII     M  E  T          T  G  E  I  E  L            BglII
             Kappa Leader                   aa23
```

Lambda chain insertion region of pAc-λ-Fc

```
                                           SacI         EcoRV
agatctagcatggcctgg..45bp..gcccagtctgtggagctccagccggatatcagatct
BglII    M  A  W          A  Q  S  V  E  L           BglII
            Lambda Leader                  aa23
```