Product datasheet

Baculo Expression Vector pAc-lambda-Fc

Short overview

Cat. No.	PR3003
Quantity	5 µg
Concentration	0.5 µg/µl in TE buffer

Product description

Host	E. coli
Purity	OD 260/280 ratio: 1.8-2.0
Quality check	Efficiency in standard transformation procedure with chemically competent E. coli cells (XL1-Blue) > 1E+08 cfu/µg DNA
Application	Cloning of heavy and light chain variable domain genes of scFv antibody fragments selected from hybridomas, single B-lymphocytes or phage display libraries; Expression of immunoglobulin heavy and light chains in insect cells and secretion of assembled IgG(kappa) or IgG(lamba) antibodies into the supernatant
Stability	Minimum 1 year when stored at -20°C
Vector	pAcUW51 derivative, AmpR, 6701 bp
Cloning site	XhoI and SpeI for heavy chain Fab fd genes, SacI and EcoRV for light chain Fab (lambda) genes
Purification	Plasmid Purification Kit
Storage	-20°C
Intended use	Research use only

Background

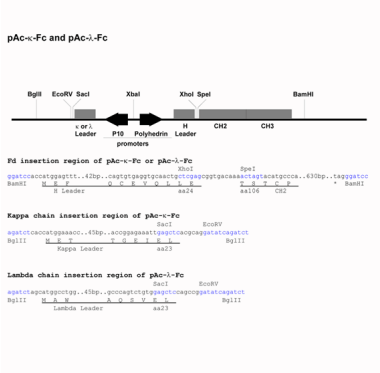
IntroductionRecombinant antibody technologies have been widely used to produce various single-chain Fv or Fab antibody fragments of different specificity. The randomized combination of cloned variable heavy and light chain immunoglobulin gene fragments further allowed the construction of human antibody 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 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 (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 becomes obsolete with the use of PROGEN's 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.Vector DesignTo 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 (BD Biosciences), 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-kappa-CH3 and

pAc-lambda-CH3 the recognition sites of the restriction endonucleases XhoI and NheI 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-kappa-Fc and pAc-lambda-Fc were instead designed for the insertion of a Fab fd gene fragment using the recognition sites of the restriction endonucleases XhoI and SpeI. The light chain gene cassette elements, starting with the authentic signal sequence of the human kappa chain in pAc-kappa-CH3 and pAc-kappa-Fc or lambda chain in pAc-lambda-CH3 and pAc-lambda-Fc, were placed in opposite orientation to the heavy chain operon under control of the p10 promotor.The recognition sites of the restriction endonucleases SacI and HindIII allow the insertion of a VL gene fragment into pAc-kappa-CH3 and pAc-lambda-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-kappa-Fc and pAc-kappa-Fc were designed for the insertion of a Fab light chain gene fragment using the recognition sites of the restriction endonucleases SacI and EcoRV (Liang et al., 2001).Material Required - Equipment for DNA cloning

- Basic cell culture equipment
- Insect cell line Sf9
- BaculoGold DNA, Transfection buffer A and B set (BD Biosciences)

Preparation of ReagentsThe 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.Cloning procedureThe 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-kappa-CH3 or pAc-lambda-CH3, the recognition sites of the restriction endonucleases XhoI and NheI are recommended. The corresponding VL gene fragment should be introduced by using the recognition sites of the enzymes SacI and HindIII. To clone a Fab fd gene fragment into pAc-kappa-Fc or pAc-lambda-Fc, the recognition sites of the restriction endonucleases XhoI and SpeI 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 SacI and EcoRV. 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-kappa-Fc or pAc-lambda-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 Duebel., 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. Baculovirus transfectionWe recommend to use the baculovirus transfection kit from BD Biosciences providing linearized and modified AcNPV baculovirus DNA (BaculoGold DNA). The antibody genes supplied on PROGE's transfer vector are flanked by regions homologous to AcNPV. When the linearized 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 Duebel., 2001; Liang et al., 2001).

Product images



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