

Product datasheet

pSEX81 Surface Expression Phagemid Vector

Short overview

Cat. No.	PR3005
Quantity	5 µg
Concentration	0.5 µg/µl in TE buffer

Product description

Host	E. coli lacIq genotype (e.g. XL1-Blue)
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
Stability	Minimum 1 year when stored at -20°C
Vector	4883 bp, AmpR
Cloning site	MluI and NotI for light chain VL genes, NcoI and HindIII for heavy chain VH genes
Note	For plating we recommend to use LB Agar plates containing 100 mM Glucose
Purification	Plasmid Purification Kit
Storage	-20°C
Intended use	Research use only

Background

Introduction Within the past decade, recombinant antibody technologies have been widely used to produce various single-chain Fv antibody fragments of different specificity. The randomized combination of PCR-amplified immunoglobulin variable heavy and light chain genes further led to the construction of large human or mouse scFv antibody libraries. The isolation of high affinity antibodies derived thereof was established by the expression of recombinant antibody fragments on the surface protein pIII of M13 bacteriophages, which can be rapidly screened against antigens bound to a solid phase.

The M13 bacteriophage protein pIII is located at one end of its tubular virion structure. It is a relative flexible and accessible molecule composed of two functional domains: an N-terminal domain that binds to the F pilus of bacteria during infection and a C-terminal domain buried within the virion. ScFv antibody fragments can be inserted near its N-terminus without destroying its function.

PROGEN now offers the phagemid cassette vector pSEX81 for the expression of functional single-chain Fv antibody - pIII fusion proteins on the surface of M13 bacteriophages. The phagemid was specifically constructed for cloning of immunoglobulin heavy and light chain gene fragments isolated from human or mouse antibody libraries generated with PROGEN's PCR primer sets F2000 or F2010.

Vector Design

The cassette vector pSEX81 was designed for the convenient insertion of heavy and light chain variable domain coding regions and for production of functional single-chain Fv antibody - pIII fusion proteins on the surface of M13 bacteriophages. The corresponding DNA fragments of human or mouse origin can be amplified by PCR using PROGEN's primer sets F2000 or F2010, respectively. The amplified gene fragments encoding the variable heavy or light chain domain are cloned in-frame between a signal peptide sequence of bacterial pectate lyase (pelB) for the secretion of the fusion protein into the periplasmic space, and the pIII gene of M13 bacteriophage. The VH and VL genes were joined by a DNA-fragment

coding for a flexible 18 amino acid residue linker containing the first six amino acids of the CH1 constant region domain and the hydrophilic pig brain alpha-tubulin peptide sequence EEGEFSEAR. The vector backbone further provides a strong promotor, the T7 terminator, the ColE1 origin of replication, the intergenic region of phage F1 and an ampicillin resistance marker for selection.

In pSEX81 the recognition sites of the restriction endonucleases Nco I and Hind III allow the insertion of a VH gene fragment. For insertion of a VL gene fragment the sites of the restriction endonucleases Mlu I and Not I are recommended.

Material Required

Equipment for DNA cloning

Preparation of Reagents

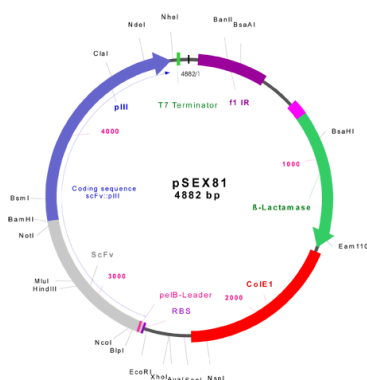
The DNA should be diluted and transferred into E. coli cells as described in standard protocols. Use an overnight culture of a single clone to extract enough DNA for the following cloning procedures.

Cloning procedure

The immunoglobulin heavy and light chain Fv domain coding regions must be cloned in-frame into pSEX81 (see attached graphic). To clone VH gene fragments the recognition sites of the restriction endonucleases Nco I and Hind III are recommended. VL gene fragments should be introduced by using the recognition sites of the enzymes Mlu I and Not I. The cloning sites of the selected Fv gene fragments have to match the corresponding amino acid positions given by the vector. Gene fragments derived from human or mouse origin with PROGEN's PCR primer sets F2000 or F2010 can be directly cloned into pSEX81.

After cloning, the vector is ready-to-use for the expression of functional recombinant single-chain Fv antibody - pIII fusion proteins in E. coli. If the modified pSEX81 is co-transfected with M13 bacteriophage as described in standard protocols (Schmiedl et al., 2000; Schmiedl and Duebel, 2001), the scFv antibody - pIII fusion protein is expressed on the surface of resulting phage particles. For expression of scFv antibody libraries on the surface of M13 bacteriophages, we recommend to use the M13 Hyperphage from PROGEN (Cat. No. PRHYPE).

Product images



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References

Publication	Species	Application
Hlaing, S. T., Srimanote, P., Tongtawe, P., Khantisitthiporn, O. & Glab-ampai, K. Isolation and Characterization of scFv Antibody against Internal Ribosomal Entry Site of Enterovirus A71. (2023).		
Bortot, B. et al. Advanced photodynamic therapy with an engineered M13 phage targeting EGFR: Mitochondrial localization and autophagy induction in ovarian cancer cell lines. Free Radic. Biol. Med. 179, 242â€“251 (2022).		
Van Fossen, E. M. et al. Creating a Selective Nanobody Against 3-Nitrotyrosine Containing Proteins. Front. Chem. 10, 1â€“13 (2022).		
Dolgikh, V. V. et al. Construction of scFv Antibodies against the Outer Loops of the Microsporidium Nosema bombycis ATP/ADP-Transporters and Selection of the Fragment Efficiently Inhibiting Parasite Growth. Int. J. Mol. Sci. 23, (2022).		
Kraivong, R. et al. Cross-reactive antibodies targeting surface-exposed non-structural protein 1 (NS1) of dengue virus-infected cells recognize epitopes on the spaghetti loop of the Î²-ladder domain. PLoS One 17, (2022).		