invitrogen

pDisplay™ Mammalian Expression Vector

PRODUCT INFORMATION SHEET

Catalog Number V66020

Publication Number MAN0025665

Revision A.0

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, California 92008 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0025665

Revision	Date	Description
A.0	27 September 2021	• Updated product form from lyophilized to liquid solution in TE Buffer. • Renamed "Maintenance of pDisplay™" to "Competent E. Coli". • Initial release with new publication number format. • Supersedes publication number 110810 28-0148 Rev. D. • Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

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1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Invitrogen™ pDisplay™ Mammalian Expression Vector is a 5.3 kb mammalian expression vector that allows display of proteins on the cell surface. Proteins expressed from the pDisplay™ vector are fused at the N-terminus to the murine Ig κ-chain leader sequence and at the C-terminus to the platelet derived growth factor receptor (PDGFR) transmembrane domain. The N-terminus to the murine Ig κ-chain leader sequence directs the protein to the secretory pathway. The C-terminus to the PDGFR transmembrane domain anchors the protein to the plasma membrane, displaying it on the extracellular side. Recombinant proteins expressed from the pDisplay™ vector contain the hemagglutinin A and myc epitopes for detection by western blot or immunofluorescence. To get started with cloning into the pDisplay™ vector, see "Guidelines for cloning" on page 7.

The pDisplay™ Mammalian Expression Vector has been used to express c-Jun and a single chain antibody against phOx hapten. Both proteins were shown to be correctly expressed at the cell surface by incubation with anti-Myc antibody followed by incubation with a magnetic bead-conjugated secondary antibody. Transfected cells were selected by using a magnet.

Contents and storage

Catalog numbers that appear as links open the web pages for those products.

Plasmids are shipped at room temperature. Upon receipt, store the plasmid as indicated.

Contents	Cat. No.	Amount	Storage
pDisplay™ Mammalian Expression Vector	V66020	20 µg in TE Buffer, pH 8.0	-20°C

Recommended materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Product	Quantity	Cat. No.
Myc Tag Monoclonal Antibodies, one of the following (or equivalent):		
Myc Tag Monoclonal Antibody	50 µL	R950-25
Myc Tag Monoclonal Antibody, HRP	50 µL	R951-25
Myc Tag Monoclonal Antibody, AP	50 µL	R952-25
Competent *E. coli*[1]		
One Shot™ BL21 Star™ (DE3) Chemically Competent *E. coli*	21 x 50 µL	C601003
BL21 Star™ (DE3)pLysS One Shot™ Chemically Competent *E. coli*	21 x 50 µL	C602003
BL21-AI™ One Shot™ Chemically Competent *E. coli*	21 x 50 µL	C607003
One Shot™ TOP10F' Chemically Competent *E. coli*	21 x 50 µL	C303003
Geneticin™ antibiotics		
Geneticin™ Selective Antibiotic (G418 Sulfate), Powder	1 g	11811023
Geneticin™ Selective Antibiotic (G418 Sulfate), Powder	5 g	11811031

[1] Chemically-competent and electrocompetent TOP10F' cells are available from **www.thermofisher.com**.

Product qualification

The pDisplay™ Mammalian Expression Vector (Cat. No. V66020) is qualified by restriction digest. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The following table lists the restriction enzymes used to digest the vector and the expected fragments.

Restriction enzyme	Expected fragments
*Bam*H I	170 bp 5,155 bp
Bgl II	5,325 bp
Pst I	5,325 bp

2 Guidelines and recommendations

Guidelines for cloning

This section provides general guidelines for cloning your gene of interest into the pDisplay™ Mammalian Expression Vector.

The pDisplay™ Mammalian Expression Vector is a fusion vector requiring that you clone your gene of interest in frame with the initiation ATG of the N-terminal Ig κ-chain leader sequence and the C-terminal myc epitope/PDGFR-TM. It may be necessary to use PCR to create a fragment with the appropriate restriction sites to clone in frame at both ends. Carefully inspect your gene and the multiple cloning site before cloning your gene of interest (see Figure 1).

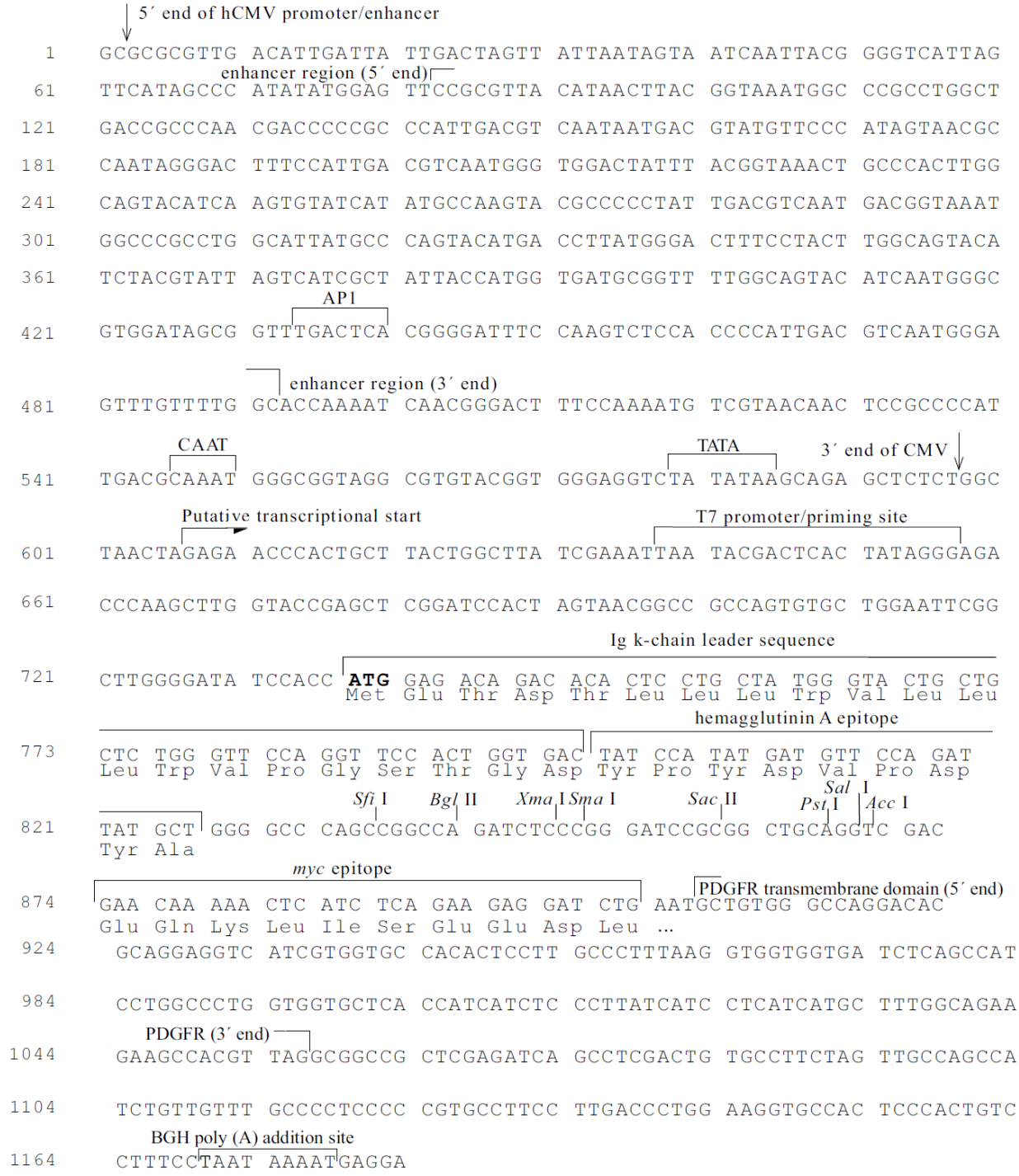

Figure 1 Multiple cloning site of the pDisplay™ Mammalian Expression Vector

Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.

Guidelines for transformation and transfection

This section provides general guidelines and recommendations for transformation and transfection.

Before you begin

E. coli transformation

- Transform your ligation mixtures into a competent recA, endA E. coli strain (e.g., TOP10, TOP10F´, DH5α), then select on LB plates containing 50–100 µg/mL ampicillin. Select 10–20 clones, then analyze for the presence and orientation of your insert.
- We recommend that you sequence your construct with the T7 Promoter Primer (Cat. No. N56002) and a gene specific reverse primer to confirm that your gene is correctly fused to the Ig κ-chain leader sequence at the N-terminus and the myc/PDGFR-TM peptide at the C-terminus.

Plasmid preparation

Once you have confirmed that your gene is correctly fused, prepare plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipids, decreasing transfection efficiency. We recommend using the S.N.A.P.™ Plasmid DNA MiniPrep Kit (Cat. No. K190001) for isolation of 10–15 µg of plasmid DNA or CsCl gradient centrifugation for isolation of >15 µg of plasmid DNA.

Recommended methods

For established cell lines (e.g., HeLa, 293), consult the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells.

Guidelines for Geneticin™ selection

Geneticin™ antibiotic blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn5, in mammalian cells results in detoxification of Geneticin™ antibiotic.

- Prepare Geneticin™ antibiotic in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Calculate the concentration based on the amount of active drug (check the lot label).
- Use 100 – 800 µg/ml of Geneticin™ antibiotic in complete medium.
- Test varying concentrations of Geneticin™ antibiotic on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin™ antibiotic.

Cells will divide once or twice in the presence of lethal doses of Geneticin™ antibiotic, so the effects of the drug take several days to become apparent. Complete selection can take up to 3 weeks of growth in selective medium.

Recommended methods to detect displayed proteins

To confirm that your protein is displayed on the cell membrane, we recommend the following methods:

- *In situ* immunofluorescent labeling of cells with antibodies to c-Myc
- *In situ* immunofluorescent labeling of cells with antibodies to hemagglutinin A
- An indirect magnetic selection procedure with a magnetic bead-conjugated secondary antibody

Troubleshooting

Observation	Possible cause	Recommended action
Protein is not displayed	Protein is not in frame with PDGFR-TM.	Use antibody to hemagglutinin or myc to check for expression.
		Sequence your construct to check frame.
No expression	Method of transfection is not optimal.	Optimize transfection or change transfection method.
	Protein is not in frame with Ig k-chain leader sequence.	Sequence your construct to check frame.

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Supplemental information

Features of the pDisplay™ Mammalian Expression Vector

pDisplay™ Mammalian Expression Vector (5,325 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein.
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
ATG initiation codon	Permits initiation of translation of the pDisplay™ vector fusion protein.
Murine Ig κ-chain leader sequence	Targets protein to secretory pathway.
Hemagglutinin A epitope tag (Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala)	Allows detection of the fusion protein by monoclonal antibody 12CA5.
Multiple cloning region with eight unique sites	Allows insertion of your gene and facilitates cloning.
Myc epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of the pDisplay™ vector fusion protein with the anti-Myc antibodies.
Platelet-derived growth factor receptor transmembrane domain (PDGFR-TM)	Anchors the fusion protein to the plasma membrane for display.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC origin	High-copy number replication and growth in *E. coli*.
SV40 early promoter and origin	Permits expression of the kanamycin resistance gene for Geneticin™ antibiotic resistance in mammalian cells. Allows episomal replication in cells containing SV40 large T antigen.
Kanamycin resistance gene	Confers resistance to Geneticin™ antibiotic in mammalian cells.
TK polyadenylation signal	Efficient transcription termination and polyadenylation of kanamycin resistance gene mRNA.

(continued)

Feature	Benefit
Ampicillin resistance gene (β-lactamase)	Selection in *E. coli*.
f1 origin	Allows rescue of single-stranded DNA.

Map of pDisplay™ Mammalian Expression Vector

The following map summarizes the features of the pDisplay™ Mammalian Expression Vector. For the complete sequence of the vector, go to **www.thermofisher.com** or contact Technical Support (see "Customer and technical support" on page 16). Details of the multiple cloning site are shown on page 11

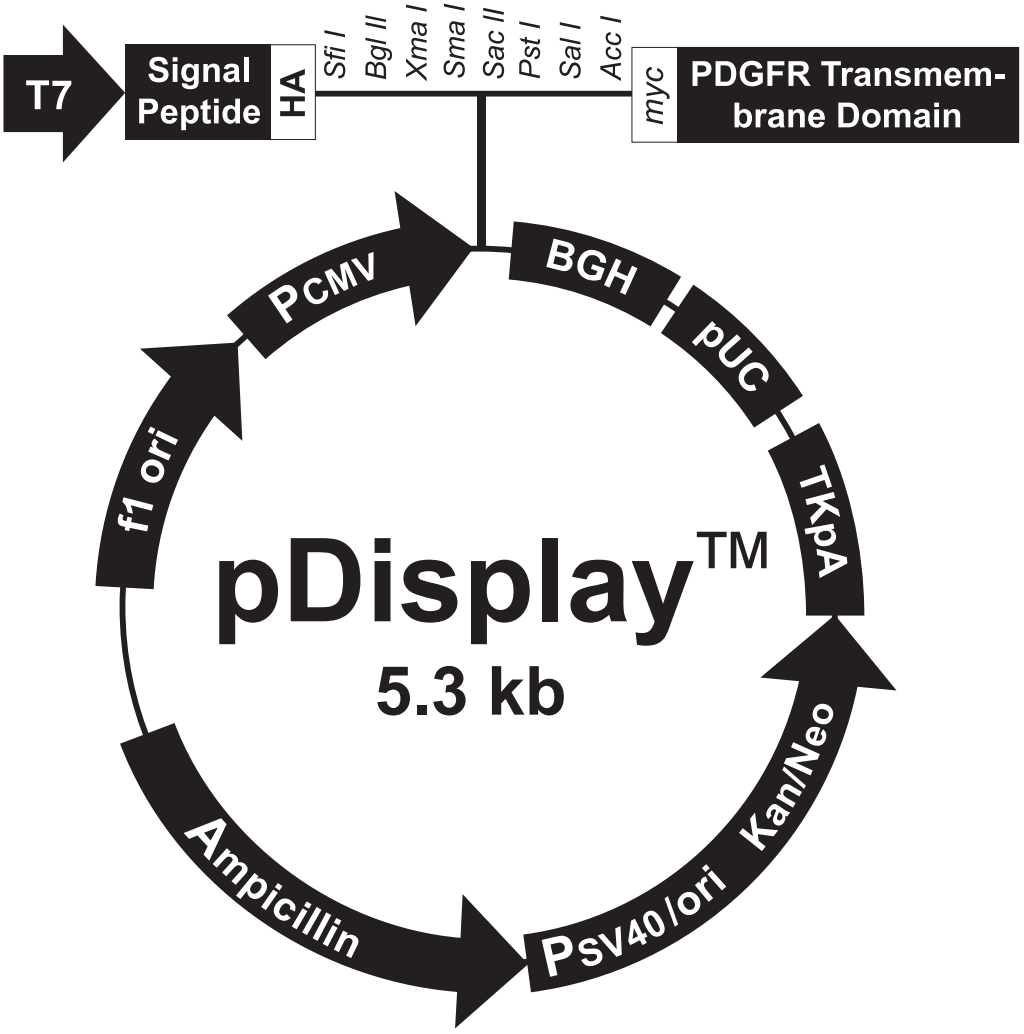

Figure 2 Map of pDisplay™ Mammalian Expression Vector Vector

Comments for the pDisplay™ Mammalian Expression Vector: 5325 necleotides

① CMV promoter: bases 1-596
② T7 promoter: bases 638-657
③ Murine Ig kappa-chain V-J2-C signal peptide: bases 737-799
④ Hemagglutinin A epitope: bases 800-826
⑤ Multiple Cloning Site: bases 827-873
⑥ *myc* epitope: bases 874-903
⑦ PDGFR transmembrane domain: bases 907-1056
⑧ Bovine growth hormone polyadenylation signal: bases 1069-1288
⑨ pUC origin: bases 1378-2051
⑩ Thymidine kinase polyadenylation site: bases 2458-2187
⑪ Neomycin/Kanamycin resistance gene: bases 3421-2366
⑫ SV40 origin and promoter: bases 3797-3456

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
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  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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