

Product Information

pPolh-FLAG™-1 Transfer Vector

Catalog Number **T6824**Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

pPolh-FLAG-1 is a 5575 bp baculovirus transfer vector used for producing FLAG® fusion proteins in insect cells. N-terminal FLAG-tagged fusions are created by cloning a properly inserted open reading frame into the multiple cloning site (MCS). The pPolh-FLAG-1 vector contains the strong viral polyhedrin (polh) promoter for high-level expression of target genes during the very late phase of infection. The vector also contains a high copy bacterial origin of replication and an ampicillin resistance gene (amp^r) for easy propagation in *Escherichia coli* host cells.

Following co-transfection with linear baculovirus DNA into insect cells, allelic replacement between homologous viral sequences (AcNPV ORF 603 and ORF 1629) in the vector and the baculovirus DNA transfers the FLAG-target gene fusion sequence into the viral genome.^{1,2} The vector is designed to be compatible with most baculoviral DNA systems that require the essential gene ORF1629 for complementation of lethal deletions and the recovery of viable recombinant virus.

The N-terminal FLAG (DYKDDDDK) fusion proteins may be detected using the ANTI-FLAG® M2 monoclonal antibody (Product Number F 3165) and purified using the ANTI-FLAG M2 Affinity Gel (Product Number A 2220). Sigma-Aldrich also offers a wide selection of related ANTI-FLAG products; please visit www.sigma-aldrich.com for a complete listing of antibody conjugates, resins and affinity capture plates.

The following table provides map positions to key features in the pPolh-FLAG-1 Transfer Vector. Sequence verification of the MCS can be performed using the following recommended primers from Sigma-Genosys.

N-Terminal Junction:

5'- CCATCTCGCAAATAAATAAGTA -3'

C-Terminal Junction:

5'-CTGTAAATCAACAACGCACAG-3'

pPolh-FLAG-1 Features

Feature	Map Position
AcNPV sequence (ORF 603)	1-1146
Recommended 5' primer sequence binding site	1079-1100
polh Promoter	1076-1145
FLAG	1164-1187
MCS	1188-1246
Recommended 3' primer sequence binding site	1300-1320
M13 origin	2576-3229
polyA	1599-1604
AcNPV sequence (ORF1629)	1286-2629
β -lactamase (amp^r)	3616-4473
pUC ori	4624-5267

Reagents

- pPolh-FLAG-1 Transfer Vector, 20 μg , T 6824, 0.5 mg/ml in 10mM Tris-HCl, pH 8.0, 1mM EDTA.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Product ships on dry ice. Store at -20°C .

References

1. Bishop, D. H. L., and Possee, R. D., Baculovirus expression vectors. *Advances Gene Technol.* **1**, 55-72 (1990).
2. O'Reilly, D. R., et al., *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press, NY, 1994).

The diagram illustrates the pPolh-FLAG-1 plasmid construct. At the top, a linear map shows the ATG start codon, a FLAG tag, and a Multiple Cloning Site (MCS) with restriction sites for Xho I, Not I, EcoR I, Pst I, Sma I, Kpn I, BamH I, and Bgl II. The circular plasmid map below shows the following components: a polh promoter (indicated by an arrow), a polyA signal, an AcNPV origin of replication (yellow arc), an ORF 1629 (yellow arc), an M13 Origin (teal arc), an amp^r gene (purple arc), and a pUC ori (blue arc). The plasmid is labeled pPolh-FLAG-1 in the center.

FLAG Peptide Sequence																		
Met	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Xho I		Not I		EcoR I					
ATG	GAC	TAC	AAA	GAC	GAT	GAC	GAC	AAG	CTC	GAG	GCG	GCC	GCG	AAT	TCC			
TAC	CTG	ATG	TTT	CTG	CTA	CTG	CTG	TTC	GAG	CTC	GCG	CGG	GCG	TTA	AGG			

Pst I				Sma I				Kpn I		BamH I		Bgl II	
TGC	AGT	CAA	CGC	GTC	CCG	GGG	GTA	CCG	GAT	CCA	GAT	CT	
ACG	TCA	GTT	GCG	CAG	GGC	CCC	CAT	GGC	CTA	GGT	CTA	GA	

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