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Product Information

pPolh-FLAG™-2 Transfer Vector

Catalog Number **E6155**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

pPolh-FLAG-2 is a 5573 bp baculovirus transfer vector used for producing FLAG® fusion proteins in insect cells. C-terminal FLAG-tagged fusions are created by cloning a properly inserted open reading frame having no stop codons into the multiple cloning site (MCS). Target gene sequences require an ATG translational initiator sequence, and optimum expression is obtained if the ATG initiator is in a proper "Kozak-like" context.[1] The pPolh-FLAG-2 vector contains the strong viral polyhedrin (polh) promoter for high-level expression of target genes in 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 target gene-FLAG fusion sequence into the viral genome.[2,3] 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 C-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 offers a wide selection of related 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-2 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-2 Features

Feature	Map Position
AcNPV sequence (ORF 603)	1-1146
Recommended 5′ primer sequence binding site	1079-1100
polh Promoter	1076-1145
MCS	1148-1211
FLAG	1212-1235
Recommended 3' primer sequence binding site	1298-1318
M13 origin	2574-3227
polyA	1597-1602
AcNPV sequence (ORF1629)	1284-2627
β-lactamase (amp^r)	3614-4471
pUC ori	4622-5265

Reagents Provided

- pPolh-FLAG-2 Transfer Vector, 20 µg, E 6155, 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM 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

This product ships on dry ice and storage at –20 °C is recommended.

References

1. Kozak. M., Initiation of translation in prokaryotes and eukaryotes. Gene, **234,** 187-208 (1999).
2. Bishop, D. H. L., and Possee, R. D., Baculovirus expression vectors. *Advances Gene Technol.* **1,** 55-72 (1990).
3. O'Reilly, D. R., et al., *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press, NY, 1994).

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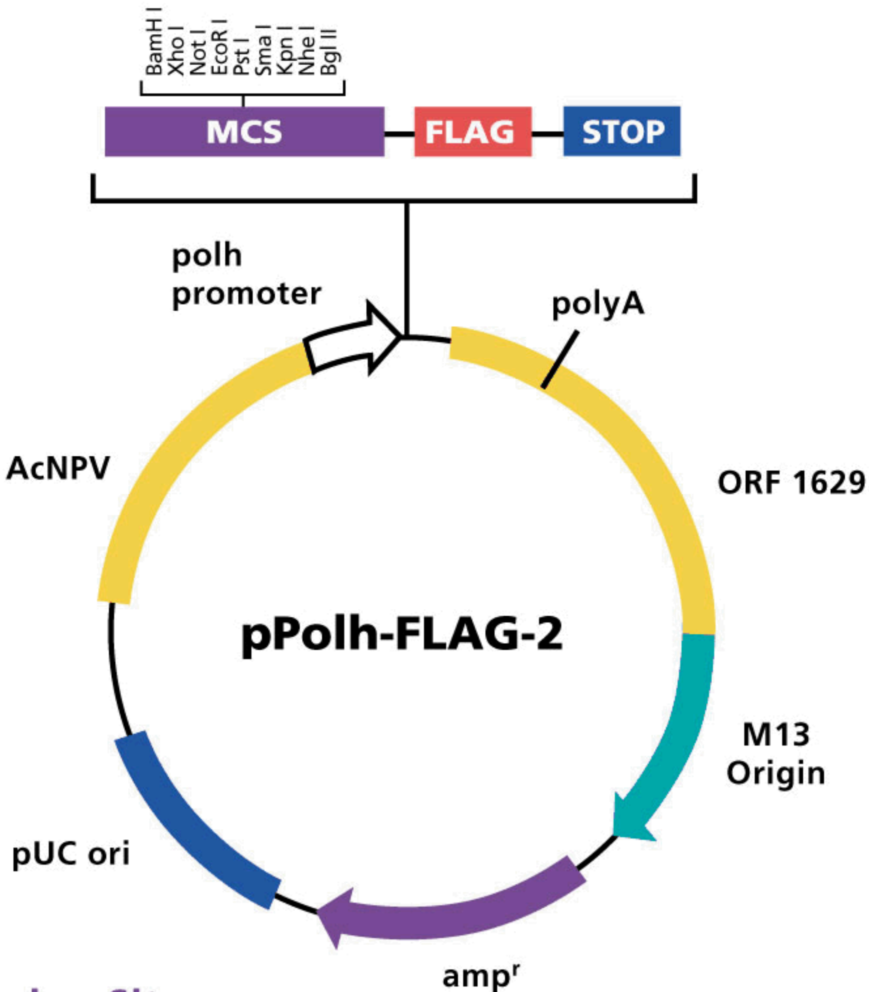
pPolh-FLAG-2 (5.6 kb)



Multiple Cloning Site
(pPolh-FLAG-2)