

Product Information

pPolh-MAT-Tag™-1 Transfer Vector

Catalog Number **T6699**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

pPolh-MAT-Tag-1 is a 5575 bp baculovirus transfer vector used for producing Metal Affinity Tag (MAT-Tag) fusion proteins in insect cells. N-terminal MAT-Tagged fusions are created by cloning a properly inserted open reading frame into the multiple cloning site (MCS). The pPolh-MAT-Tag-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 MAT-Tag 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 MAT-Tag amino acid sequence has transition metal, e.g., Ni^{+2} and Co^{+2} , binding properties that may be exploited for purification. N-terminal MAT-Tag (HNHRHKH) fusion proteins may be purified using HIS-Select® Nickel Affinity Gel, Catalog Number P6611. Sigma-Aldrich offers a wide variety of related HIS-Select products; please visit www.sigma-aldrich.com for a complete listing of affinity conjugates, resins and capture plates.

Reagent

- pPolh-MAT-Tag-1 Transfer Vector, 20 μg , 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.

Vector Features

The following table provides map positions to key features in the pPolh-MAT-Tag-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'

Feature	Map Position
AcNPV sequence (ORF 603)	1-1146
Recommended 5' primer sequence binding site	1079-1100
polh Promoter	1076-1145
MAT-Tag	1167-1187
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

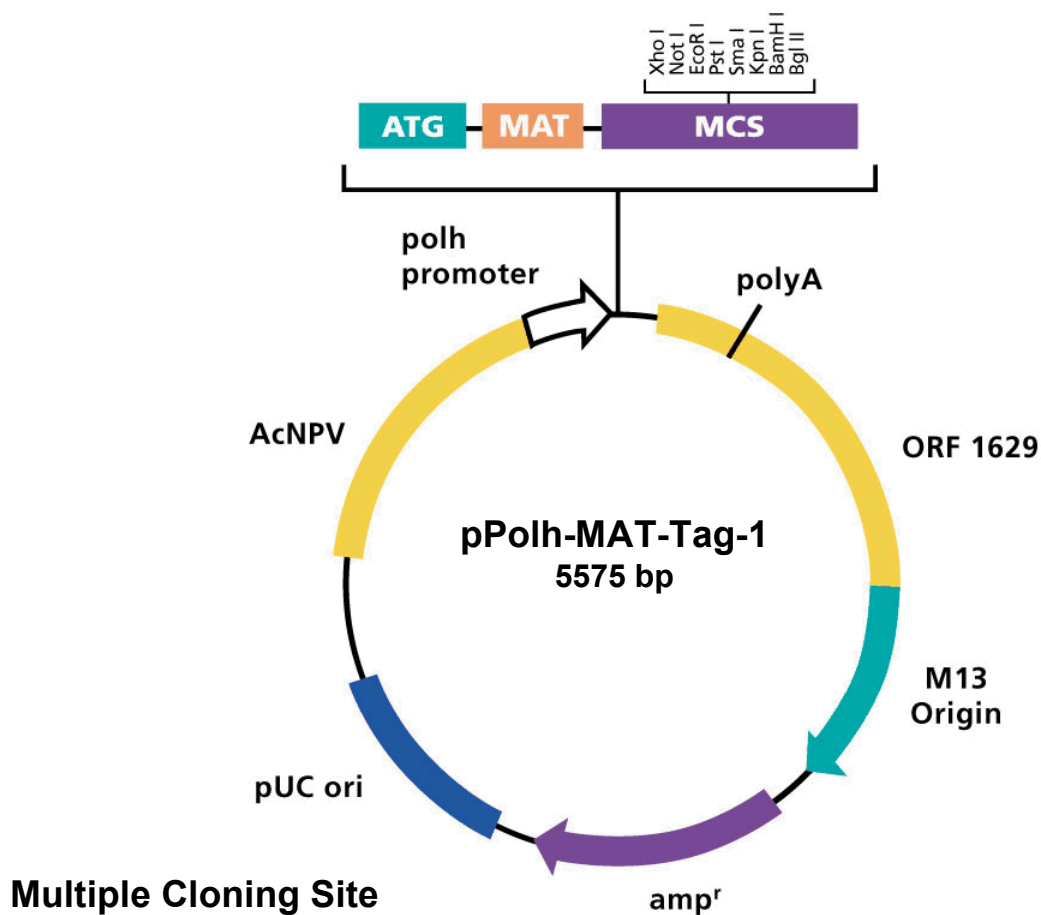
References

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* (W. H. Freeman & Co., NY, 1992).

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AH,RS,PHC 09/10-1



MAT Sequence																			
Met	Ala	His	Asn	His	Arg	His	Lys	His		Xho I	Not I	EcoR I							
ATG	GCT	CAC	AAC	CAC	CGC	CAC	AAG	CAC	CTC	GAG	GCG	GCC	GCG	AAT	TCC				
TAC	CGA	GTG	TTG	GTG	GCG	GTG	TTC	GTG	GAG	CTC	CGC	CGG	CGC	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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