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Human PMEL cDNA Clone in Bacterial Expression Vector (His tag)

Overview	
Quantity:	500 ng
Gene:	Melanoma gp100 (PMEL)
Species:	Human
Fusion tag:	His tag
Insert:	cDNA
Vector:	Bacterial Expression Vector
Application:	Cloning (Clon)
Product Details	
Purpose:	Bacterial expression of Human SILV with His tag
Insert Length:	1986 bp
Vector Backbone:	pPB-N-His
Promoter:	T7 Promoter
Bacterial Resistance:	Kanamycin
Expression Type:	Transient
Specificity:	5-Nhel and 3-Xhol
	Fusion tag: A singel N-terminal 6X-Histidine tag which is cleavable with Thrombin (Size 2.3 kDa)
Sequencing Primer:	T7 promoter primer: 5'-TAATACGACTCACTATAGGG-3', T7 terminator primer: 5'-
	GCTAGTTATTGCTCAGCGG-3'
Target Details	
Gene:	Melanoma gp100 (PMEL)
Alternative Name:	SILV (PMEL Products)

Application Details

Application Notes:

The pPB vectors are low-medium copy number vectors in which the gene expression is driven by the strong T7 promoter.

Below are some basic guidelines for using the pPB vectors for protein production:

- 1. The pPB vectors are designed to be used with E. coli strains that are DE3 lysogens i.e. the host E. coli cell has a source of T7 RNA polymerase.
- 2. Recombinant protein induction is usually done at OD600 of 0.6-1.2 using Isopropyl β -D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.05 -1mM.
- 3. The ideal concentration of IPTG must be determined empirically for each recombinant protein/cell-line. Similarly, the length of time and temperature for induction provide other variables that need to be optimized on a case-to-case basis.
- 4. For toxic proteins, it is recommended to go for shorter induction time and also to try and suppress basal recombinant gene expression through (a) addition of glucose or use of pLysS plasmid. Please note that special cell-lines are also available in the market that cater to expression of toxic proteins.
- 5. Once grown for the desired length of time, harvest cells by centrifugation and either freeze the cells at -80°C (as such or after re-suspending in the desired buffer) or proceed with the purification.

Restrictions:

For Research Use only

Handling

Format:	Liquid
Buffer:	10 mM Tris-HCI, 1 mM EDTA, pH 8.0
Storage:	-20 °C
Storage Comment:	1 year when stored at -20° C or lower in a non-frost free freezer.
Expiry Date:	12 months

Publications

Product cited in:

Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (1991)