

pVectOZ-SEAP (Secreted Alkaline Phosphatase Expression Vector)

Description

The pVectOZ-SEAP vector has been created to produce the highest levels of Secreted Alkaline Phosphatase expression in a broad range of mammalian cells and tissues. It contains a proprietary modified human cytomegalovirus (CMV) promoter followed by a specific intron, enhancer and a terminator. The expression vector is engineered in an optimized plasmid backbone to achieve the highest levels of transgene expression in mammalian cells and high copy number production in *Escherichia coli*.

Kit contents

Ref. #PL00150
100µg pVectOZ-SEAP (encoding for Secreted Alkaline Phosphatase) plasmid in 100µl sterile TE buffer.

Storage

Store at -20°C.

Selection Marker

Kanamycin is the selection gene included for producing the plasmid in *Escherichia coli*.

Applications

pVectOZ-SEAP (Secreted Alkaline Phosphatase) vector is suitable for all transfection applications (*in vitro* & *in vivo*).

Presentation. The transgene expression level depends mainly on the promoter, enhancer, terminator and plasmid backbone. The pVectOZ-SEAP expression cassette was designed to express very high levels of transgene product in many mammalian cells and tissues. This vector has been modified to eliminate sequences affecting transgene expression levels while optimizing those critical for high levels of expression. The final expression cassette accommodates the high levels of transgene expression in mammalian cells as well as high yield of plasmid production in *Escherichia coli*. The resulting plasmid is the ideal vector to reach high levels of expression *in vitro* and *in vivo*.

Use. For high levels of transgene expression in mammalian cells and tissues. For optimal results, this vector can be used with all OZ Biosciences transfection reagents to transfect a wide variety of mammalian cells and tissues.

SEAP detection

48 hours after transfection, collect the supernatants from transfected cells and heat them at 65°C for 30 minutes to inactivate endogenous alkaline phosphatase activity. The SEAP activity is quantitatively measured by using a colorimetric assay based on hydrolysis of the chromogenic substrate para-nitrophenyl phosphate (PNPP). 1 mg/ml of PNPP reagent is prepared in 1mM MgCl₂, 1M Diethanolamine, pH 9.8. Then, 10 µl of 0.05% Zwittergent® in PBS (Ca²⁺ and Mg²⁺ free) is added to each well of a 96-well plate, and mixed with 20 µL of the heated cell culture media. For control wells, 20 µL of water is used to normalize the volume. An alkaline phosphatase (AP) standard (EIA grade calf intestine alkaline phosphatase) can be used to generate a standard curve from 1 to 100 pg per well. Thereafter, the enzymatic reaction starts by adding, 200 µL of the PNPP substrate to each well and incubate at room temperature for 5-45 minutes prior to analysis. The use of 0.05% Zwittergent® in PBS as the diluent nearly reduces the background to zero, which increases the sensitivity of the assay. The plates are read at 405 nm using either kinetic or static settings.

References

1. De Jong, AS. *et al.* (1985). *Histochem J.* **17**: 1119.
2. Yang, TT. *et al.* (1997). *Biotechniques.* **23**: 1110.
3. Kain, SR. *et al.* (1997). *Methods Mol Biol.* **63**: 49.
4. Liu, M. *et al.* (2003). *Methods Mol Biol.* **235**: 289.
5. Chalberg, T. *et al.* (2005). *Encyclopedia Life Sciences.*
6. Ufer, C. *et al.* (2008). *Gene Dev.* **22**: 1838.

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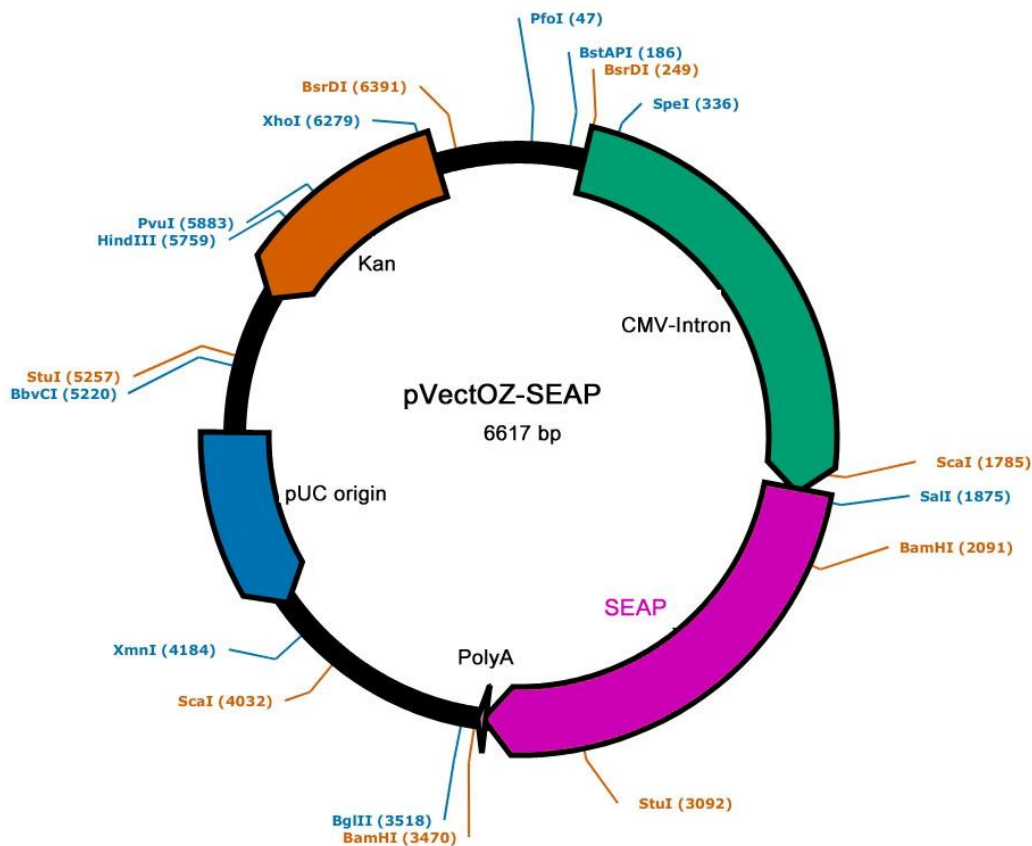


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Plasmid Map



Related Products

Description	Reference	Description	Reference
Magnetofection Technology		Gene & Protein Tools	
Mega Magnetic Plate	MF14000	Bradford – Protein Assay Kit	BA00100
Super Magnetic Plate	MF10000	GeneBlaster selection kit	GB20010
Magnetic Plate 96-magnets	MF10096	β-Galactosidase (ONPG) assay kit	GO10001
PolyMag 1 mL (for all nucleic acids)	PN31000	β-Galactosidase (CPRG) assay kit	GC10002
PolyMag Neo 1 mL (for all nucleic acids)	PG61000	X-Gal Staining Kit	GX10003
CombiMag 1 mL (boost transfection reagent)	CM21000	Plasmids	
SilenceMag 500µL (for siRNA applications)	SM10500	pVectOZ-CAT 25µg	PL00010
NeuroMag 1 mL (for neuron transfection)	NM51000	pVectOZ-GFP 25µg	PL00020
		pVectOZ-LacZ 25µg	PL00030
		pVectOZ-Luc 25µg	PL00040
		pVectOZ-SEAP 25µg	PL00050
Lipofection (lipid-based reagents)		pVectOZ-CAT 100µg	PL00110
DreamFect Gold Transfection reagent 1 mL	DG81000	pVectOZ-GFP 100µg	PL00120
DreamFect Transfection reagent 1 mL	DF41000	pVectOZ-LacZ 100µg	PL00130
Lullaby siRNA Transfection reagent 1 mL	LL71000	pVectOZ-Luc 100µg	PL00140
VeroFect Transfection Reagent 1 mL	VF61000		
FlyFectin Transfection Reagent 1 mL	FF51000		