Cat. # 3243
3244

For Research Use

TaKaRa

pBApo-EF1α Neo DNA
pBApo-EF1α Pur DNA

Product Manual

v201612Da

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I. Description

pBApo-EF1α is a simple gene expression vector for mammalian cells. This vector carries a promoter from human polypeptide chain elongation factor (EF-1α promoter) and a polyA signal site from herpes simplex virus thymidine kinase gene. The vectors are useful for the construction of expression plasmids by inserting the ORF of a target gene at the multicloning site. This vector can also be used to express microRNA precursors and other transcripts in addition to ordinary genes.

The pBApo-EF1α series includes vectors carrying a neomycin-resistance gene or a puromycin-resistance gene as a selection marker in mammalian cells.

II. Product Information

pBApo-EF1α Neo DNA (Cat. #3243)	20 μg
pBApo-EF1α Pur DNA (Cat. #3244)	20 μg

Concentration : 0.5 μg/μl
Form : 10 mM Tris-HCl, pH8.0, 1 mM EDTA

III. Storage

−20℃
2 years from date of receipt under proper storage conditions.

IV. Vector Map and Cloning Sites

EcoR V
Cla I
EcoR I
SV40 polyA
EF1α promoter
BamH I
Xba I
Sse8387 I
Hind III
HSV TK polyA
EcoR V
Cla I
NeoR
SV40 promoter
AmpR

pBApo-EF1α Neo
5,167 bp

EcoR V
Cla I
EcoR I
SV40 polyA
EF1α promoter
BamH I
Xba I
Sse8387 I
Hind III
HSV TK polyA
EcoR V
Cla I
PurR
SV40 promoter
AmpR

pBApo-EF1α Pur
4,972 bp

Figure 1. Vector map

1194 1214 1234

5'-TGAAGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGGG-3'

*Bam*H I *Xba* I *Sse*8387 I *Hind* III

Figure 2. Multicloning site on pBApo-EF1α Neo and pBApo-EF1α Pur

V. Protocol

1. Gene insertion
   Insert the ORF of a target gene into the cloning site of a plasmid vector. The ampicillin resistance gene carried by the vector allows for selection of transformed *E. coli*.

2. Transfection
   Transfect plasmids using a transfection reagent such as the Xfect™ series (Cat. #631317 etc.) under the conditions specified in its protocol.

3. Transfected cell selection
   - pBApo- EF1α Neo DNA has a neomycin resistance gene and pBApo- EF1α Pur DNA has a puromycin resistance gene to allow for drug selection of transfected cells.
   - Drug selection should be started at least 24 hours after plasmid transfection. In case of a high cell density, reseed cells at appropriate dilution and replace the drug containing medium every 3 - 4 days. Generally, transfected cells can be obtained in 1 - 2 weeks.
   - Since drug sensitivity varies from cell to cell, determine ahead of time the optimum concentration for the cell used. The concentration will generally be 500 - 1,000 μg/ml of G418 for Neo^R gene and 1 - 3 μg/ml of puromycin for Pur^R gene.

VI. Experimental Examples

1. Construction of a fluorescent protein expression vector (AcGFP1)

- After digestion of pBApo-EF1α Neo DNA with *Hin*d III, cleaved ends were blunted and further digestion with *Xba* I was carried out. Subsequently, an approximately 5.1 kb DNA fragment was recovered by agarose gel electrophoresis.
- A DNA fragment of AcGFP1 gene removed from pAcGFP1-C1 Vector (Cat. #632470) by *Nhe* I and *Ssp* I restriction digestions and the digested pBApo-EF1α Neo were ligated using the DNA Ligation Kit <Mighty Mix> (Cat. #6023).
- *E. coli* JM109 Competent Cells (Cat. #9052) were transformed with the ligation mix and plated on LB plates containing ampicillin.
- The colonies obtained were cultured in 2 - 5 ml of LB Amp liquid medium to prepare plasmids.
- One of the prepared plasmids was transfected into cultured HeLa cells using Xfect Transfection Reagent (Cat. #631317).
- Cells were observed 24 hours later using a fluorescence microscope to verify the expression of AcGFP1. (Figure 3)

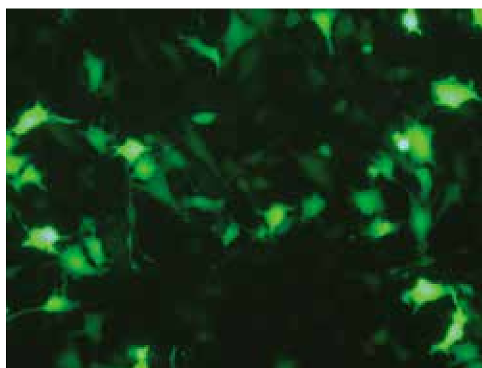

Figure 3. Fluorescence microscopy: 24 hours after transfection of pBApo-EF1α Neo / AcGFP1

2. Comparative expression between EF1α promoter and CMV-IE promoter in mouse ES cells

- Mouse ES E14TG2a cells were transfected with a plasmid (pBApo-EF1α Neo or pBApo-CMV Neo) carrying the AcGFP1 gene using Xfect mESC Transfection Reagent (Cat. #631320).
- Cells were recovered after 48 hours and analyzed for AcGFP1 expression using a flow cytometer (Figure 4. Transient expression).
- Transfected cells were selected by using medium containing 250 μg/ml G418 and analyzed for AcGFP1 expression using a flow cytometer (Figure 4. Stable expression).

EFp: pBApo-EF1α Neo / AcGFP1 — % Positive: the percentage of AcGFP1-positive cells
CMVp: pBApo-CMV Neo / AcGFP1 — MFI: mean fluorescent intensity of positive cells

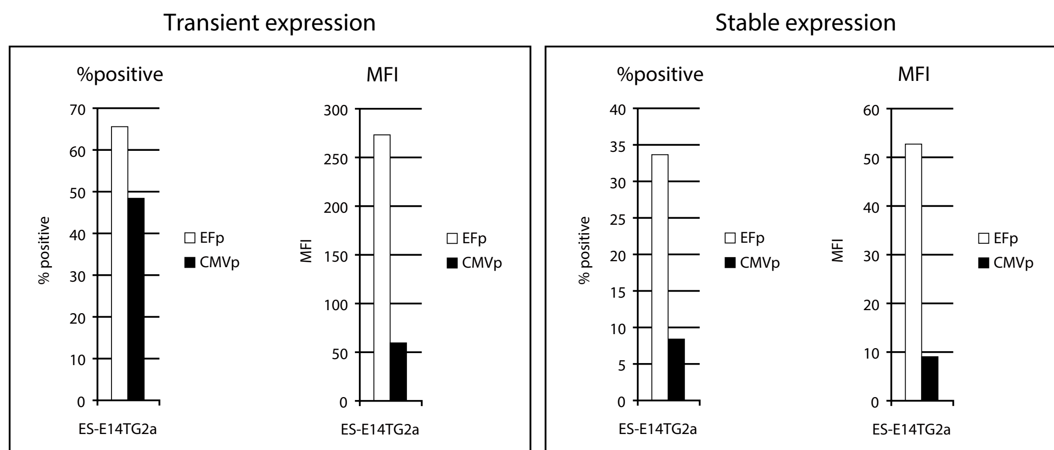

Figure 4. Flow cytometer analyses of transfected cells

Result : The EF1α promoter provided a higher percentage of AcGFP1-positive cells and a higher level of transient and stable expression in mouse ES cells.

VII. Related Products

pBApo-CMV Vector Series (Cat. #3240_3242)
DNA Ligation Kit < Mighty Mix> (Cat. #6023)
E. coli JM109 Competent Cells (Cat. #9052)
Xfect™ Transfection Reagent (Cat. #631317)
Xfect™ mESC Transfection Reagent (Cat. #631320)
pAcGFP1-C1 Vector (Cat. #632470).
G418 (Cat. #631307, 631308)
Puromycin (Cat. #631305, 631306)

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