Cat. # 3390
3392

For Research Use

TaKaRa

pHEK293 Ultra Expression Vector I
(Cat. #3390)

pHEK293 Ultra Expression Vector II
(Cat. #3392)

Product Manual

v201612Da

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I. Description

The pHEK293 Ultra Expression Vectors are plasmid vectors developed for transient overexpression of recombinant proteins in human HEK293 cells. Protein expression using these vectors is 2 - 10 times higher than what is obtained from vectors with conventional cytomegalovirus (CMV)-derived promoters.

- The pHEK293 Ultra Expression Vector I is a single plasmid for overexpression a protein of interest in HEK293 cells.
- The pHEK293 Ultra Expression Vector II is co-transfected with the pHEK293 Enhancer Vector, allowing the expression level of the target protein to be optimized by varying ratio of the two plasmids.

(pHEK293 Ultra Expression Vector I)

The pHEK293 Ultra Expression Vector I includes the CMV IE promoter derived from the cytomegalovirus, the Trans-Activation-Responsive region (TAR), a multiple cloning site (MCS), an internal ribosome entry site (IRES), a transactivator (Tat) gene, and the herpes simplex virus thymidine kinase poly-A signal (HSV TK polyA) (see Figure 4). Transient overexpression of the target protein is possible through insertion of the gene of interest (GOI) into the MCS and transfection of HEK293 cells with the recombinant plasmid.

(pHEK293 Ultra Expression Vector II)

The pHEK293 Ultra Expression Vector II includes the CMV IE promoter derived from the cytomegalovirus, TAR, a multicloning site (MCS), and a herpes simplex virus thymidine kinase poly-A signal (HSV TK polyA) (see Figure 5). The pHEK293 Enhancer Vector contains the Tat gene (see Figure 6.) Transient overexpression of the target protein is possible through insertion of the GOI into the MCS of the pHEK293 Ultra Expression Vector II and cotransfection of HEK293 cells with both the recombinant expression vector and the pHEK293 Enhancer Vector. It is possible to vary the ratio of the pHEK293 Ultra Expression Vector II and pHEK293 Enhancer Vector used for cotransfection to modulate the expression level of the target protein.

Note: These vectors are optimized for overexpression in HEK293 cells; sufficient overexpression may not be obtained in cell types other than human cells.

II. Components

pHEK293 Ultra Expression Vector I (Cat. #3390)	
1. pHEK293 Ultra Expression Vector I	20 μg (1 μg/μl)
pHEK293 Ultra Expression Vector II (Cat. #3392)	
1. pHEK293 Ultra Expression Vector II	20 μg (1 μg/μl)
2. pHEK293 Enhancer Vector	20 μg (1 μg/μl)

III. Storage

-20℃

Use within 2 years of receipt.

IV. Principle

The pHEK293 Ultra Expression Vectors include TAR, an RNA sequence derived from HIV-1 virus, and the transcription activator Tat (i.e., TAR-Tat expression system; Figure 1). Tat is an RNA-binding protein that activates transcription in HIV-1 by binding to the transactivation response element (TAR) stem loop structure that forms at the 5′ end of HIV RNAs. Binding of Tat to TAR promotes transcriptional elongation.

The pHEK293 Ultra Expression Vectors exploit this transcriptional activation mechanism; the TAR sequence is present in the 5' untranslated region of the target gene, and a Tat expression cassette is included, either on the same vector (Vector I, Figure 2) or on a separate plasmid (Vector II, Figure 3). This system allows efficient expression of Tat protein, and thereby it is possible to greatly increase the amount of target protein expressed (Figures 2 and 3; patent pending). Since the TAR sequence is located in the untranslated region of the target gene, it does not have an effect on the amino acid sequence of the expressed target protein.

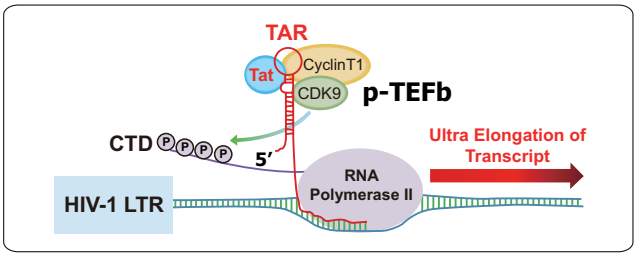


Figure 1. Principles of the TAR-Tat expression system

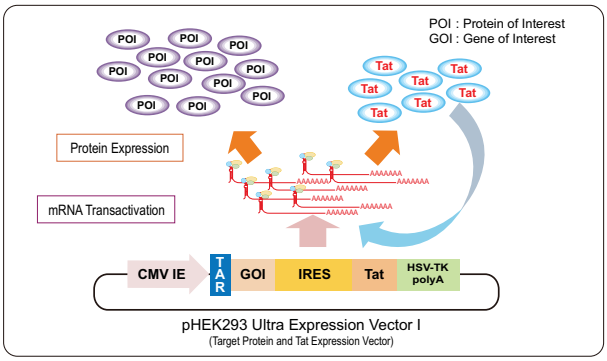


Figure 2. Protein overexpression Using pHEK293 Ultra Expression Vector I

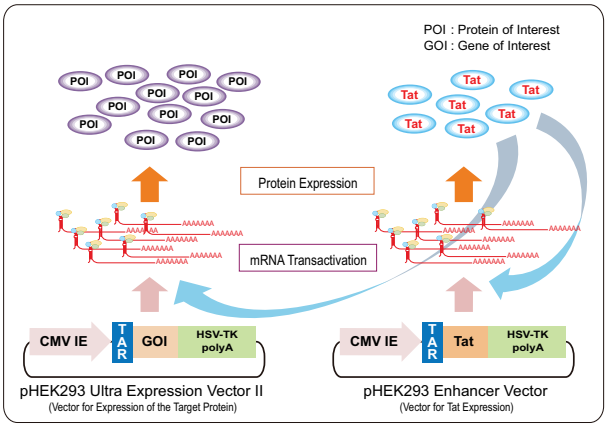


Figure 3. Protein overexpression using pHEK293 Ultra Expression Vector II

V. Vector Maps

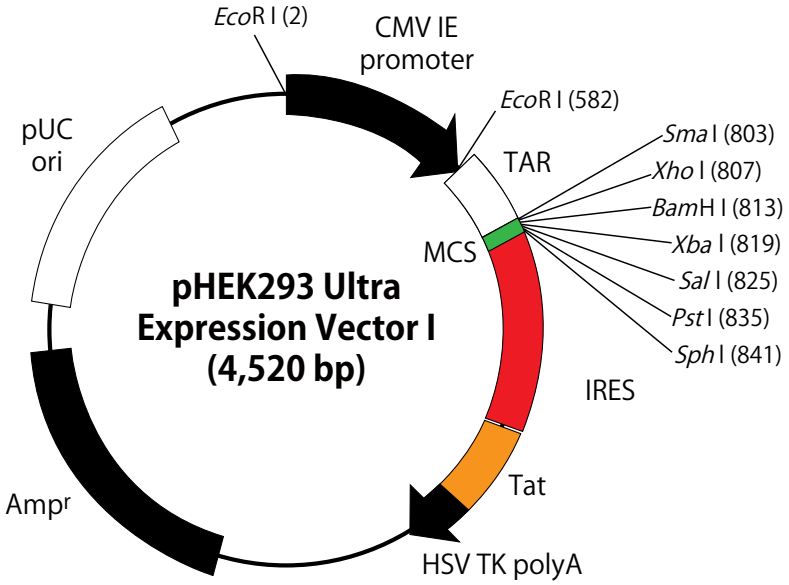
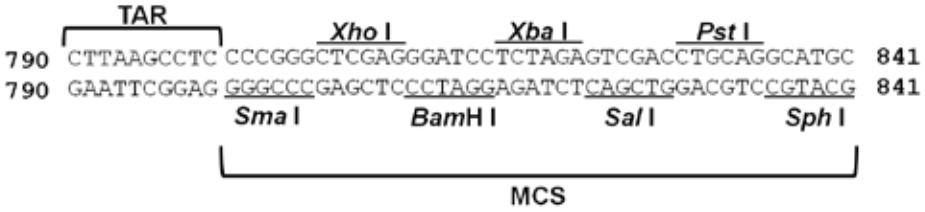


Figure 4. Vector Map of pHEK293 Ultra Expression Vector I



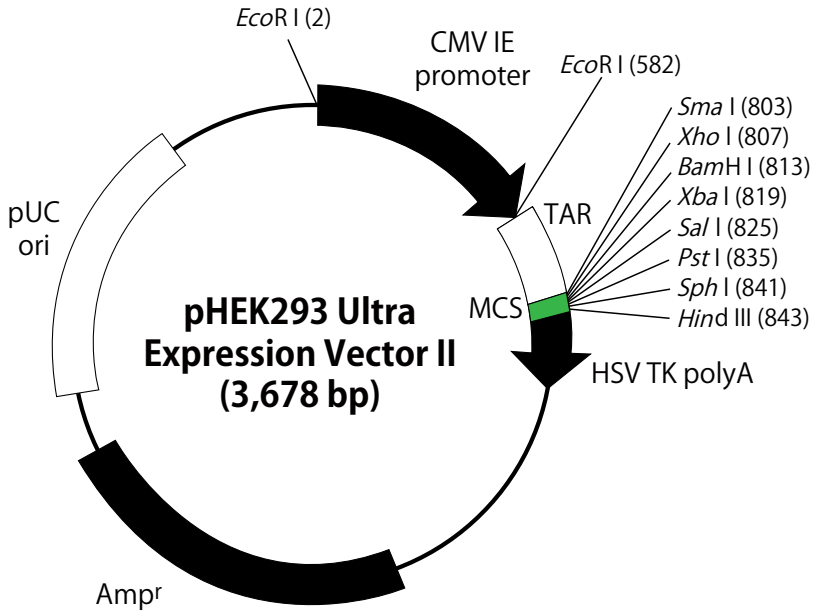
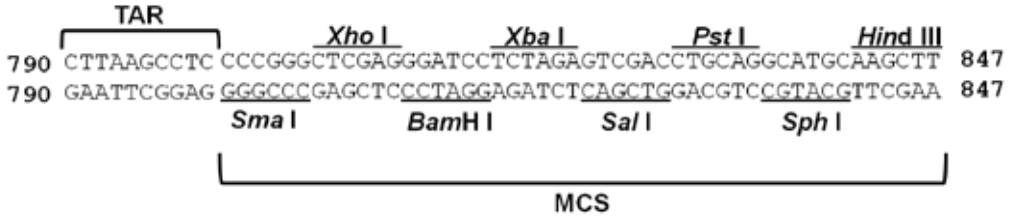


Figure 5. Vector Map of pHEK293 Ultra Expression Vector II



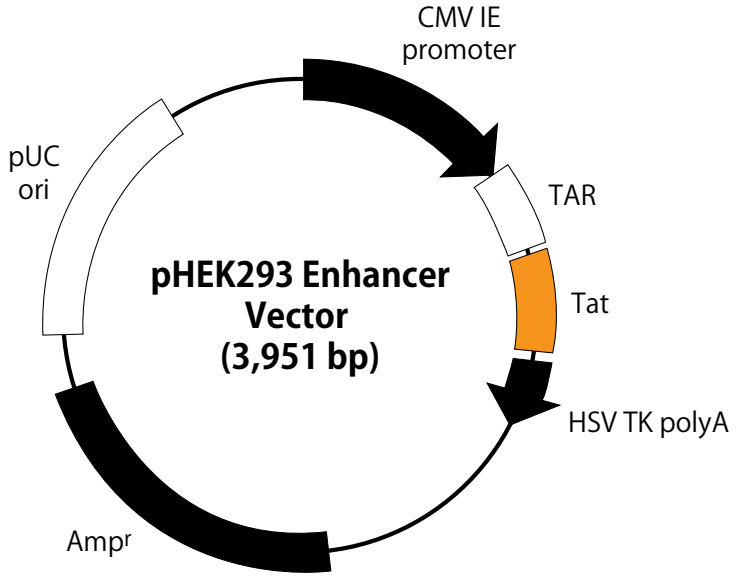


Figure 6. Vector Map of pHEK293 Enhancer Vector

VI. Protocol

It may be necessary to modify culture conditions depending on the cells used and target protein being expressed. A general protocol is provided below.

VI-1. Insertion of a Gene of Interest (GOI)

Clone the ORF of a GOI into the MCS of pHEK293 Ultra Expression Vector I or pHEK293 Ultra Expression Vector II using standard cloning methods. The GOI sequence (cDNA or gene fragment) should include both start and stop codons. The In-Fusion® HD Cloning Kit Plus (Cat. #638909, etc.) can be used for cloning. The In-Fusion system allows PCR products to be easily cloned into any type of linear vector. When cloning, use competent cells that have a reduced risk of recombination, such as *E. coli* HST08 Premium Competent Cells (Cat. #9128) or Stellar™ Competent Cells (Cat. #636763). Recombinant *E. coli* can be selected by ampicillin resistance.
After identifying clones, prepare plasmids that are of suitable purity for transfection of cultured human cells. NucleoBond Xtra Midi or Midi Plus (Cat. #740410.10 or 740412.10, etc.) is recommended for plasmid purification.

VI-2. Transfection into Cells

Transfect HEK293 cells with the recombinant plasmid using a commercially-available transfection reagent. Use the methods and amount of plasmid according to the protocol for the transfection reagent being used.

[When Using pHEK293 Ultra Expression Vector II]

When using pHEK293 Ultra Expression Vector II, cotransfection with pHEK293 Enhancer Vector is necessary. It is possible to modify the ratio of the vectors based on the efficiency of the target protein expression. A ratio of 5 : 1 (by weight) of Ultra Expression Vector II to pHEK293 Enhancer Vector is recommended.

VII. Experimental Examples

VII-1. Expression of a Fluorescent Protein (AcGFP1)

VII-1-1. Suspension HEK293 cells (FreeStyle 293-F cells, Thermo Fisher Scientific) cultured in a 125-ml flask were transfected according to the recommended protocol (293fectin Transfection Reagent, Thermo Fisher Scientific). The plasmids used in each experiment are shown in Table 1.

Table 1. Expression vectors.

Exp.	AcGFP1 Expression Vector		pHEK293 Enhancer Vector
	Plasmid	Amount (μg)	Amount (μg)
1	pBApo-CMV/AcGFP1	30	-
2	Vector I/AcGFP1	30	-
3	Vector II/AcGFP1	30	0.24
4	Vector II/AcGFP1	30	6
5	Company A Vector/ AcGFP1	30	-

pBApo-CMV : pBApo-CMV DNA (Cat. #3242)
Vector I : pHEK293 Ultra Expression Vector I
Vector II : pHEK293 Ultra Expression Vector II
Company A Vector : High-expression vector from Company A

VII-1-2. Two days after transfection, GFP expression was analyzed using fluorescence microscopy and flow cytometry (Figure 7). The fluorescence intensity was much higher for GFP expressed from the pHEK293 Ultra Expression Vectors as compared to expression from the pBApo-CMV vector or the high-expression vector from Company A (7 - 11 times and 5 - 7 times, respectively).

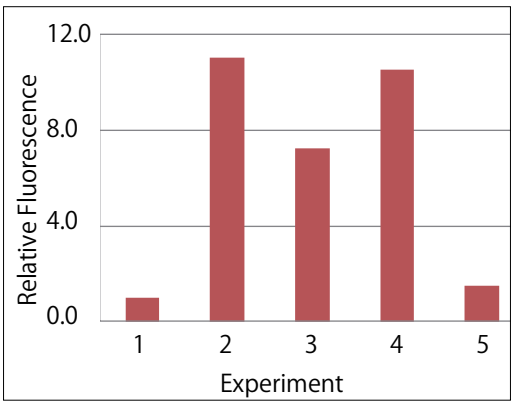


Figure 7. AcGFP1 expression.

VII-2. Expression of Granulocyte Colony Stimulating Factor (G-CSF)

VII-2-1. Suspension HEK293 cells (FreeStyle 293-F cells, Thermo Fisher Scientific) cultured in a 125-ml flask were transfected according to the recommended protocol (293fectin Transfection Reagent, Thermo Fisher Scientific). The plasmids used in each experiment are shown in Table 2.

Table 2. Expression vectors.

Exp.	G-CSF Expression Vector		pHEK293 Enhancer Vector
	Plasmid Type	Amount (μg)	Amount (μg)
1	pBApo-CMV/G-CSF	30	-
2	Vector I/G-CSF	30	-
3	Vector II/G-CSF	30	0.24
4	Vector II/G-CSF	30	6
5	Negative Control	-	-

VII-2-2. The culture supernatant was collected 2, 5, and 7 days after transfection, and G-CSF expression was quantified using the Human G-CSF Assay Kit (IBL Code. 27131) (Figure 8). Expression with the pHEK293 Ultra Expression Vectors resulted in 4 - 5 times more G-CSF expression as compared with a vector using an CMV promoter for expression (pBApo-CMV DNA).

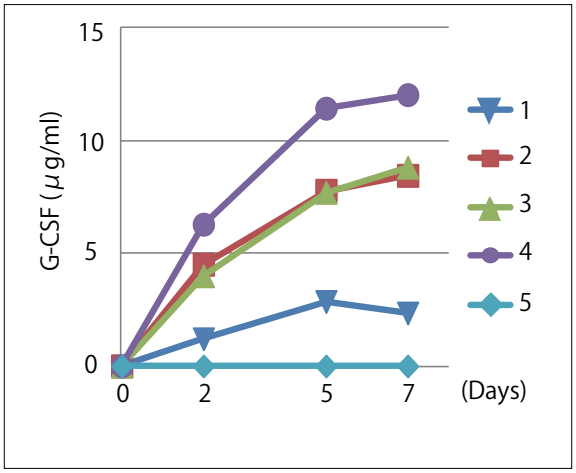


Figure 8. G-CSF expression.

VII-3. Production of Mouse Anti-Human IgG Antibody

VII-3-1. Suspension HEK293 cells (FreeStyle 293-F cells, Thermo Fisher Scientific) cultured in a 125-ml flask were transfected according to the recommended protocol (293fectin Transfection Reagent, Thermo Fisher Scientific). The plasmids used in each experiment are shown in Table 3.

Table 3. Expression vectors.

Exp.	Heavy Chain Expression Vector		Light Chain Expression Vector		pHEK293 Enhancer Vector
	Plasmid	Amount (μg)	Plasmid	Amount (μg)	Amount (μg)
1	pBApo-CMV/HC	15	pBApo-CMV/HC	15	-
2	Vector II/HC	15	Vector II/LC	15	0.24
3	Vector II/HC	15	Vector II/LC	15	6.0
4	Negative	-	Negative	-	-

HC : Heavy Chain
LC : Light Chain

VII-3-2. The culture supernatant was collected 2, 5, 7, and 9 days after transfection, and Mouse IgG was quantified using the Mouse IgG EIA Kit. The results are shown in Figure 9. Expression with the pHEK293 Ultra Expression Vectors resulted in 6 - 7 times more mouse IgG expression as compared with a vector containing a CMV promoter for expression (pBApo-CMV).

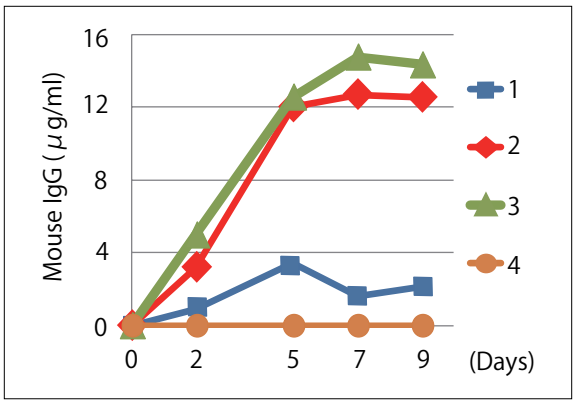


Figure 9. Mouse IgG expression.

VIII. Related Products

In-Fusion® HD Cloning Plus Kit (Cat. #638909, 638910, 638911, 638920)
E. coli HST08 Premium Competent Cells (Cat. #9128)*
Stellar™ Competent Cells (Cat. #636763)
pBApo Vector Series (Cat. #3240-3244)
NucleoBond Xtra Midi (Cat. #740410.10/.50/.100)
NucleoBond Xtra Midi Plus (Cat. #740412.10/.50)

* Not available in all geographic locations. Check for availability in your area.