# pCMV / hygro-Positive Control Vector (C-terminal Fc-His tag)



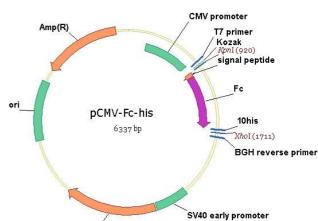
#### Catalog Number: CV008

#### **Vector Information**

- Positive control for the pCMV / hygro-HA.
- · Designed for mammalian expression, stable or transient.
- Hygromycin resistance gene for selection of stable cell lines.

### Description

Vector Name	pCMV / hygro-Positive Control Vector (C-terminal Fc-His tag)
Vector Size	6337bp
Vector Type	Mammalian Expression Vector
Expression Method	Constitutive, Stable / Transient
Promoter	CMV
Antibiotic Resistance	Ampicillin
Selection In Mammalian Cells	Hygromycin
Protein Tag	His
Sequencing Primer	Forward:T7(TAATACGACTCACTATAGGG) Reverse:BGH(TAGAAGGCACAGTCGAGG)



Hygro(R)

## pCMV / hygro-Positive Control Vector (C-terminal Fc-His tag) Sequence and Quality Control

**Physical Map** 

1	ATGGGCTGGT	CCTGCATCAT	CCTGTTCCTC	GTGGCGACCG	CGACCGGGGT	CCACAGC <sup>1</sup> GAG
61	CCCAAATCTT	CTGACAAAAC	TCACACATGC	CCACCGTGCC	CAGCACCTGA	ACTCCTGGGG
121	GGACCGTCAG	TCTTCCTCTT	ССССССАААА	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC
181	CCTGAGGTCA	CGTGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCCGAGGT	CAAGTTCAAC
241	TGGTACGTGG	ACGGCGTGGA	GGTGCATAAT	GCCAAGACAA	AGCCGCGGGA	GGAGCAGTAC
301	AACAGCACGT	ACCGTGTGGT	CAGCGTCCTC	ACCGTCCTGC	ACCAGGACTG	GCTGAATGGC
361	AAGGAGTACA	AGTGCAAGGT	CTCCAACAAA	GCCCTCCCAG	CCCCCATCGA	GAAAACCATC
421	TCCAAAGCCA	AAGGGCAGCC	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC	ATCCCGGGAT
481	GAGCTGACCA	AGAACCAGGT	CAGCCTGACC	TGCCTGGTCA	AAGGCTTCTA	TCCCAGCGAC
541	ATCGCCGTGG	AGTGGGAGAG	CAATGGGCAG	CCGGAGAACA	ACTACAAGAC	CACGCCTCCC
601	GTGCTGGACT	CCGACGGCTC	CTTCTTCCTC	TACAGCAAGC	TCACCGTGGA	CAAGAGCAGG
661	TGGCAGCAGG	GGAACGTCTT	CTCATGCTCC	GTGATGCATG	AGGCTCTGCA	CAACCACTAC
721	ACGCAGAAGA	GCCTCTCCCT	GTCTCCGGGT	AAAGCT <sup>2</sup> CACC	ATCACCACCA	TCATCACCAC
781	CATCAC <sup>3</sup> TAA					

1. Signal peptide

2. GCT was the nucleotide residue from the restriction site during plasmid construction, which has no influence on protein expression.

3. His Tag

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## **Detect Positive Control Vector Expression by Western Blot**

KDa	Α	В	С	D	Lanes Items	A	В	С	D
100— 70—					Sample	Fc-FLAG Transfected 293 Cell Lysate	Fc-HA Transfected 293 Cell Lysate	Fc-Myc Transfected 293 Cell Lysate	Fc-His Transfected 293 Cell Lysate
55 40					Sample Volume	5 µg/lane		1 µg/lane	
 35		Primary antibody	Commercial FLAG Tag Antibody	Anti-HA Tag Antibody (Cat# 100028- <b>MA</b> 10)	Anti-Myc Tag Antibody (Cat# 10002 <del>9 MM</del> 08D)	Commercial His Tag Antibody			
					Primary antibody Concentration	1:1000	1 µg/ml		
15				Secondary Antibody	Dylight 800-Labele	ylight 800-Labeled Antibody To Mouse IgG (H+L), at 1:7500 dilution.			
	Gel	13% SDS-PAGE reducing gel							

#### Protocol :

The 6 µg of plasmid was transfected into 20 ml of HEK293H suspension cells with Sinofection reagent (Cat# STF01). Experssion cells were cultured for 4d at 37 °C (5% CO<sub>2</sub>). The  $2 \times 10^7$  of cells were lysed in 1 ml of ice-cold modified RIPA Lysis Buffer with protease inhibitors cocktail (Sigma) by homogenization. The protein concentration of cell lysate was measured by BCA kit, and 1~5 µg of lysate were detected by western blotting using specific anti-tag antibody.

## **Plasmid Resuspended Protocol**

- 1. Centrifuge the tube for 5~10 min at 4,000 rpm.
- 2. Carefully open the tube and add 100  $\mu$ l of sterile water to dissolve the DNA.
- 3. Close the tube and incubate for 10 minutes at room temperature.
- 4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 4000 rpm.
- 5. Store the plasmid at -20 °C.

## E.coli strains for transformation (recommended but not limited)

Most commercially available competent cells are appropriate for the plasmid, e.g. TOP10, DH5a and TOP10F'.

### Storage

The lyophilized plasmid can be stored at ambient temperature for three months.