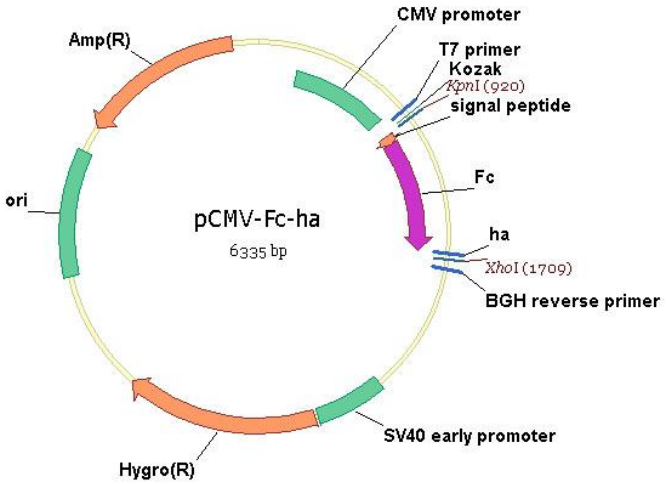
pCMV / hygro-Positive Control Vector (C-terminal Fc-HA tag)

Catalog Number: **CV007**

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.

Physical Map



Description

Vector Name	pCMV / hygro-Positive Control Vector (C-terminal Fc-HA tag)
Vector Size	6335bp
Vector Type	Mammalian Expression Vector
Expression Method	Constitutive, Stable / Transient
Promoter	CMV
Antibiotic Resistance	Ampicillin
Selection In Mammalian Cells	Hygromycin
Protein Tag	GATTACAAGGATGACGACGATAAG
Sequencing Primer	Forward:T7(TAATACGACTCACTATAGGG) Reverse:BGH(TAGAAGGCACAGTCGAGG)

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

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1       ATGGGCTGGT CCTGCATCAT CCTGTTCCTC GTGGCGACCG CGACCGGGGT CCACAGC[1]GAG
61      CCCAAATCTT CTGACAAAAC TCACACATGC CCACCGTGCC CAGCACCTGA ACTCCTGGGG
121     GGACCGTCAG TCTTCCTCTT CCCCCCAAAA 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[2]TATC CTTACGACGT GCCTGACTAC
781     GCC[3]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. HA Tag

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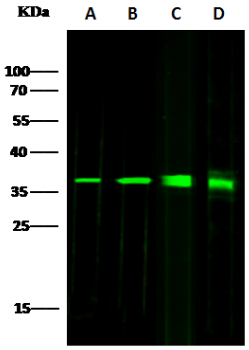
Detect Positive Control Vector Expression by Western Blot



Items \ Lanes	A	B	C	D
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
Sample Volume	5 μg/lane		1 μg/lane	
Primary antibody	Commercial FLAG Tag Antibody	Anti-HA Tag Antibody (Cat# 100028-MM10)	Anti-Myc Tag Antibody (Cat# 100029-MM08D)	Commercial His Tag Antibody
Primary antibody Concentration	1:1000	1 μg/ml		
Secondary Antibody	Dylight 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). Expersion cells were cultured for 4d at 37℃ (5% CO_2). The 2×10^7 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 μl 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 ℃.

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

Most commercially available competent cells are appropriate for the plasmid, e.g. TOP10, DH5α and TOP10F´.

Storage

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