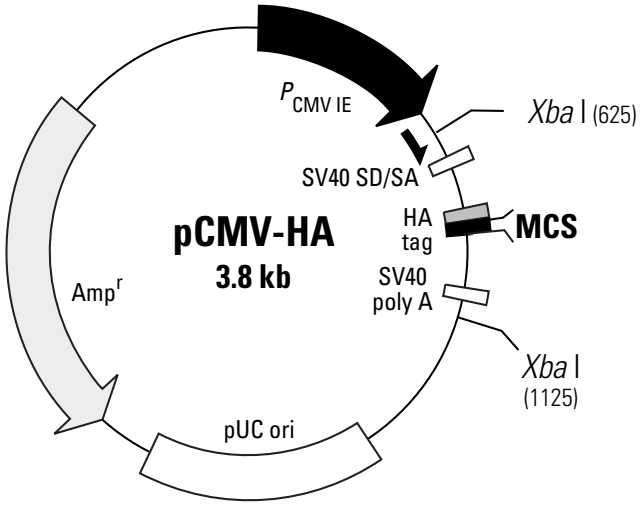
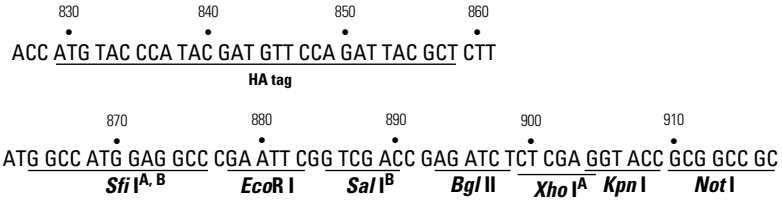
Vector Information

pCMV-HA Vector Information PT3283-5

Sold as part of Cat. No. 631604





Restriction Map and Multiple Cloning Site (MCS) of pCMV-HA. Unique restriction sites are in bold.
[A] Sites are compatible with Matchmaker™ System 3 AD Vector.
[B] Sites are compatible with Matchmaker™ System 3 BD Vector.
For older Matchmaker Systems, consult the Vector Information Packets provided with the vectors to determine compatibility.

Description:

The pCMV-HA Mammalian Expression Vector expresses proteins containing an N-terminal hemagglutinin (HA) epitope tag. The HA epitope tag is well-characterized and highly immunoreactive. High-level expression in mammalian cells is driven from the human cytomegalovirus immediate early promoter/enhancer ($P_{CMV\ IE}$). The vector contains an intron (SV40 splice donor/splice acceptor); the epitope tag; an MCS; and a polyadenylation signal from SV40. This vectors also possesses the ampicillin resistance gene for selection in *E. coli.*

Use:

To create a fusion of a gene of interest and the HA tag, insert the gene into the MCS in frame with the HA coding sequence. The resulting HA-tagged proteins can be identified with the HA-Tag Polyclonal Antibody (IgG), provided with this vector, or another antibody raised against the HA tag. The epitope tag is also useful for facilitating purification of the protein, identifying associated proteins, characterizing new proteins by immunoprecipitation, and determining subcellular localization.

The MCS in this vector is compatible with the MCSs in Clontech's Matchmaker™ Two-Hybrid System Vectors. Compatibility with System 3 Vectors is noted in the MCS diagram. Consult the Vector Information Packet provided with any Matchmaker vector for complete information.

After obtaining putative positive clones in your Matchmaker two-hybrid screen, use the pCMV-Myc and pCMV-HA Vectors to verify the interactions identified in yeast directly in mammalian cells. To accomplish this, subclone the selected inserts into the pCMV-Myc Vector and the "bait" insert into the pCMV-HA Vector. Alternatively, clone the "bait" insert into pCMV-Myc and the library inserts into pCMV-HA. To confirm predicted interactions *in vivo* via coimmunoprecipitation, cotransfect pCMV-Myc with the pCMV-HA Vector into mammalian cells and immunoprecipitate using the c-Myc Monoclonal or HA-Tag Polyclonal Antibody provided with the vectors.

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Location of features:

- Immediate early cytomegalovirus promoter ($P_{CMV\ IE}$):
  - Enhancer region: 27–431
  - TATA Box: 520–526
  - Transcription start point: 549
- Intron (SV40 splice donor/splice acceptor):
  - SV40 late 19s mRNA intron: 672–702
  - Modified SV40 late 16s mRNA intron: 672–768
- HA epitope tag with start codon (ATG): 829–858
- Multiple Cloning Site: 872–912
- SV40 polyadenylation signal:
  - Polyadenylation signal: 1044–1049
  - mRNA 3' end:1063
- pUC plasmid replication region: 1536–2179
- Ampicillin resistance (β-lactamase) gene:
  - Promoter:
    - -35 region: 3257–3252
    - -10 region: 3234–3229
  - Transcription start point: 3222
  - Ribosome binding site: 3199–3195
  - β-lactamase coding sequences:
    - Start codon (ATG): 3187–3185
    - Stop codon (TAA): 2329–2327
  - β-lactamase signal peptide: 3187–3179
  - β-lactamase mature polypeptide: 3118–2330

Sequencing primer location:

- pCMV Sequencing Primer: 631–657
  5´-GAT-CCG-GTA-CTA-GAG-GAA-CTG-AAA-AAC-3'

Propagation in *E. coli:*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.