Certificate of Analysis

iDimerize™ Inducible Homodimer Vector Set 1

Contents

Catalog No.
635064 (Not sold separately)

Lot Number
Specified on product label.

Product Information

The iDimerize Inducible Homodimer Vector Set 1 is provided in the iDimerize Inducible Homodimer System (Cat. No. 635068), which lets you to control the the homodimerization of a protein of interest in live cells via a membrane permeant compound. The iDimerize Inducible Homodimer Vector Set 1 contains two vectors encoding fusion tags that can be easily added to your protein of interest. The activity and localization of the resulting chimeric protein can be controlled by the addition of a membrane-permeant compound, B/B Homodimerizer, to the cell medium. The vector set also includes two linear selection markers for hygromycin and puromycin resistance. **NOTE:** pHom-1 and pHom-Mem1 are identical to vectors pC_4-F_V1E and pC_4M-F_V2E, respectively, previously supplied in the ARGENT Regulated Homodimerization Kit from ARIAD Pharmaceuticals, Inc.

Package Contents

- 20 µl pHom-1 Vector (500 ng/µl)
- 20 µl pHom-Mem1 Vector (500 ng/µl)
- 40 µl Linear Hygromycin Marker (50 ng/µl)
- 40 µl Linear Puromycin Marker (50 ng/µl)

Storage Conditions

- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (–70°C)

Product User Manuals

User manuals for Clontech products are available for download at **www.clontech.com/manuals**
The following user manual applies to this product:

- iDimerize Inducible Homodimer System User Manual (PT5178-1)

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pHom-1 and pHom-Mem1 Vector Information

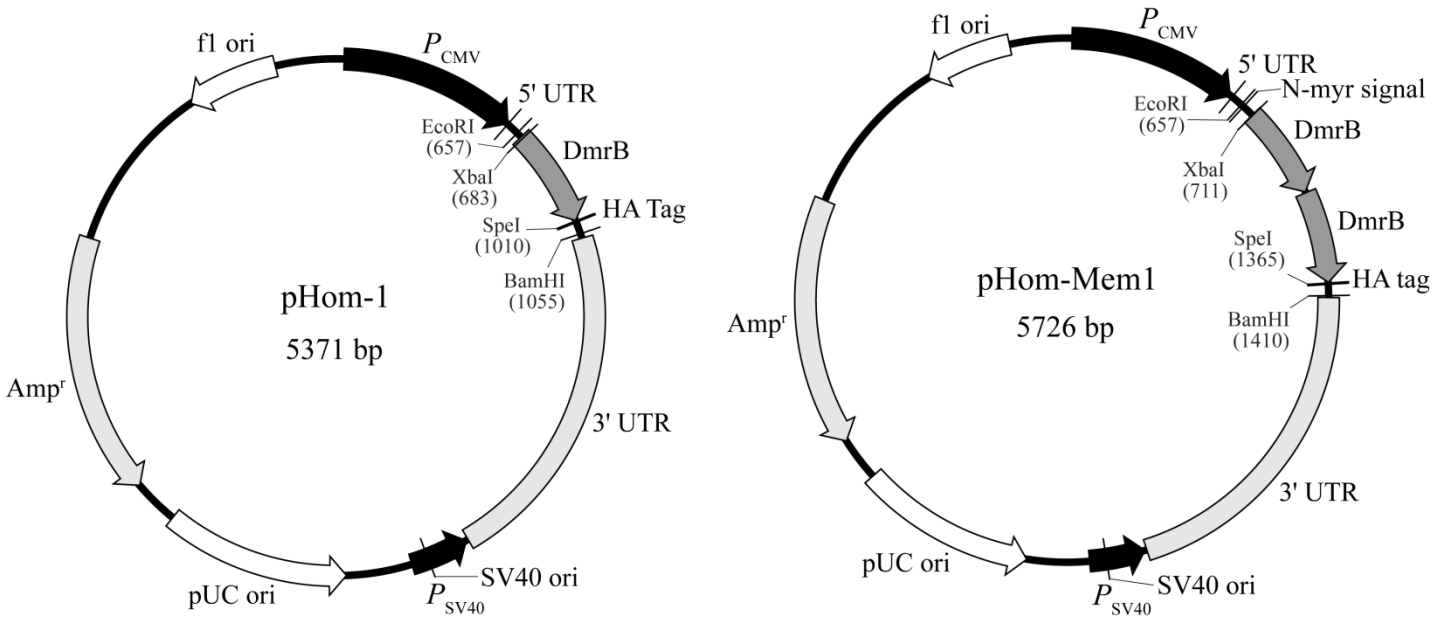

Figure 1. pHom-1 and pHom-Mem1 Vector Maps. **Cloning Strategy: For pHom-1**, use the EcoRI and XbaI sites to clone your gene of interest upstream of the dimerization domain, and the SpeI and BamHI sites to clone your gene of interest downstream of the dimerization domain. **For pHom-Mem1**, use the XbaI site to clone your gene upstream of the dimerization domains, and the SpeI and BamHI sites to clone your gene downstream of the dimerization domains. See the iDimerize Inducible Homodimer System User Manual (PT5178-1) for more information on how to clone your gene of interest. **NOTE:** In pHom-Mem1, the two DmrB-coding regions have different nucleotide sequences—they have been altered to reduce the possibility of recombination. However, both encode the same amino acid sequence.

Description

Many cellular processes are triggered by the dimerization of proteins. The vectors in this vector set allow a protein of interest to be tagged with a dimerization domain and expressed in mammalian cells. Homodimerization of the tagged protein can be induced on demand in live cells when a membrane permeant compound, the B/B Homodimerizer, is added to the tissue culture medium (1).

To induce the homodimerization of a protein of interest, it must be expressed as a fusion with the ligand-dependent homodimerization domain DmrB. In the absence of the B/B Homodimerizer compound, the fusion protein will not form homodimers. However, when the B/B Homodimerizer is added to the cell medium, the fusion protein will form a homodimeric complex. The iDimerize Inducible Homodimer Vector Set 1 contains two mammalian expression vectors that encode either one or two copies of the DmrB domain:

- **pHom-1** constitutively expresses a protein of interest fused to one copy of the dimerization domain DmrB. This vector does not contain a subcellular localization signal, so if the protein of interest does not contain a localization signal, the fusion expressed by this vector will localize to the cytosol. Ligand-induced interaction of the homodimer-tagged protein of interest will occur in the cytosol as well.
- **pHom-Mem1** constitutively express a protein of interest fused to two copies of DmrB and a myristoylation signal sequence (N-myr signal). As a result of the N-myr tag, a protein of interest expressed by the vector will be localized to the inner leaflet of the plasma membrane, and will homodimerize there upon the addition of ligand.

- The Dmr domains encoded by both vectors also include a hemagglutinin (HA) tag. This tag is useful for determining subcellular protein localization, facilitating protein purification, identifying associated proteins, and characterizing new proteins by immunoprecipitation (HA-Tag Polyclonal Antibody, Cat. No. 631207).

Both vectors drive expression of the protein of interest from the human cytomegalovirus enhancer/promoter element (P_{CMV}). To enhance expression, the vectors also contain the 5′ untranslated region (UTR) from the herpes simplex virus thymidine kinase gene, as well as a portion of the rabbit beta-globin gene 3′ UTR that includes the final intron and a polyA signal. In addition, each vector contains a pUC origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*, and an f1 origin for single-stranded DNA production.

Location of Features

pHom-1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- DmrB (dimerization domain B): 676–1008
- HA Tag (hemagglutinin epitope tag): 1015–1041
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1060–2221
- P_{SV40} (SV40 promoter): 2230–2425 (complementary)
- SV40 origin of replication: 2308–2385 (complementary)
- pUC origin of replication: 2647–3266 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 3421–4293 (complementary)
- f1 origin of replication: 4862–5168 (complementary)

pHom-Mem1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- N-myr signal (amino-terminal myristoylation signal): 668–709
- DmrB (dimerization domain B): 710–1036 & 1040–1363
- HA Tag (hemagglutinin epitope tag): 1370–1396
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1415–2576
- P_{SV40} (SV40 promoter): 2585–2780 (complementary)
- SV40 origin of replication: 2663–2740 (complementary)
- pUC origin of replication: 3002–3621 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 3776–4648 (complementary)
- f1 origin of replication: 5217–5523 (complementary)

Additional Information

See the iDimerize Inducible Homodimer System User Manual (PT5178-1) for information on how to clone your gene of interest into your vector of choice. The vectors can be transfected into mammalian cells using any standard method. Cotransfection of the vectors with Linear Hygromycin or Puromycin Markers allows antibiotic selection of stable transfectants (see user manual for details).

In the absence of the B/B Homodimerizer, the proteins tagged with DmrB will not interact with each other. The addition of the B/B Homodimerizer to the cell medium induces the interaction between the tagged proteins.

Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

1. Clackson, T. *et al.* (1998) *Proc Natl Acad Sci USA.* **95**(18):10437–10442.
2. Muthuswamy, S. K. *et al.* (1999) *Mol Cell Biol.* **19**(10):6845–6857.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzymes	Fragment Sizes
pHom-1	SpeI	5.4 kb
	EcoRI & BamHI	0.4 & 5.0 kb
pHom-Mem1	SpeI	5.7 kb
	EcoRI & BamHI	0.8 & 5.0 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

Linear Selection Marker Identity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Marker	Enzymes	Fragments
Linear Hygromycin Marker	HindIII & XbaI	0.5, 0.6 & 1.1 kb
Linear Puromycin Marker	HindIII & XbaI	0.45, 0.6, & 0.75 kb

Functional Testing of Linear Markers

- HEK 293 cells were transfected with 200 ng of either the Linear Hygromycin Marker or the Linear Puromycin Marker. After 5 hr at 37°C, the transfection solution was removed and the cells were given fresh medium. 48 hr later, the cells were plated in two 10 cm plates. 48 hr after plating, medium containing either hygromycin or puromycin was added to the plates. After 2–3 weeks, >20 clones were identified.

Notice to Purchaser

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This document has been reviewed and approved by the Clontech Quality Assurance Department.

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