iDimerize™ Inducible Heterodimer Vector Set 1

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Catalog No.
635063 (Not sold separately)

Lot Number
Specified on product label.

Product Information

The iDimerize Inducible Heterodimer Vector Set 1 is provided in the iDimerize Inducible Heterodimer System (Cat. No. 635067), which lets you control the heterodimerization of two different proteins of interest in live cells via a membrane-permeant compound. The iDimerize Inducible Heterodimer Vector Set 1 contains three vectors that provide an assortment of fusion tags (i.e., dimerization domains, an HA epitope tag, and localization sequences) that can be easily added to your proteins of interest. The activity and localization of the resulting chimeric proteins can be controlled by the addition of a small molecule (A/C Heterodimerizer) to the cell medium. The vector set also includes two linear selection markers for hygromycin and puromycin resistance. **NOTE:** pHet-1, pHet-Mem1, and pHet-Nuc1 are identical to vectors pC_4-R_HE, pC_4M-F2E, and pC_4EN-F1, respectively, previously supplied in the ARGENT Regulated Heterodimerization Kit from ARIAD Pharmaceuticals, Inc.

Package Contents

- 20 µl pHet-1 Vector (500 ng/µl)
- 20 µl pHet-Mem1 Vector (500 ng/µl)
- 20 µl pHet-Nuc1 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 Heterodimer System User Manual (PT5179-1)

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pHet-1, pHet-Nuc1 and pHet-Mem1 Vector Information

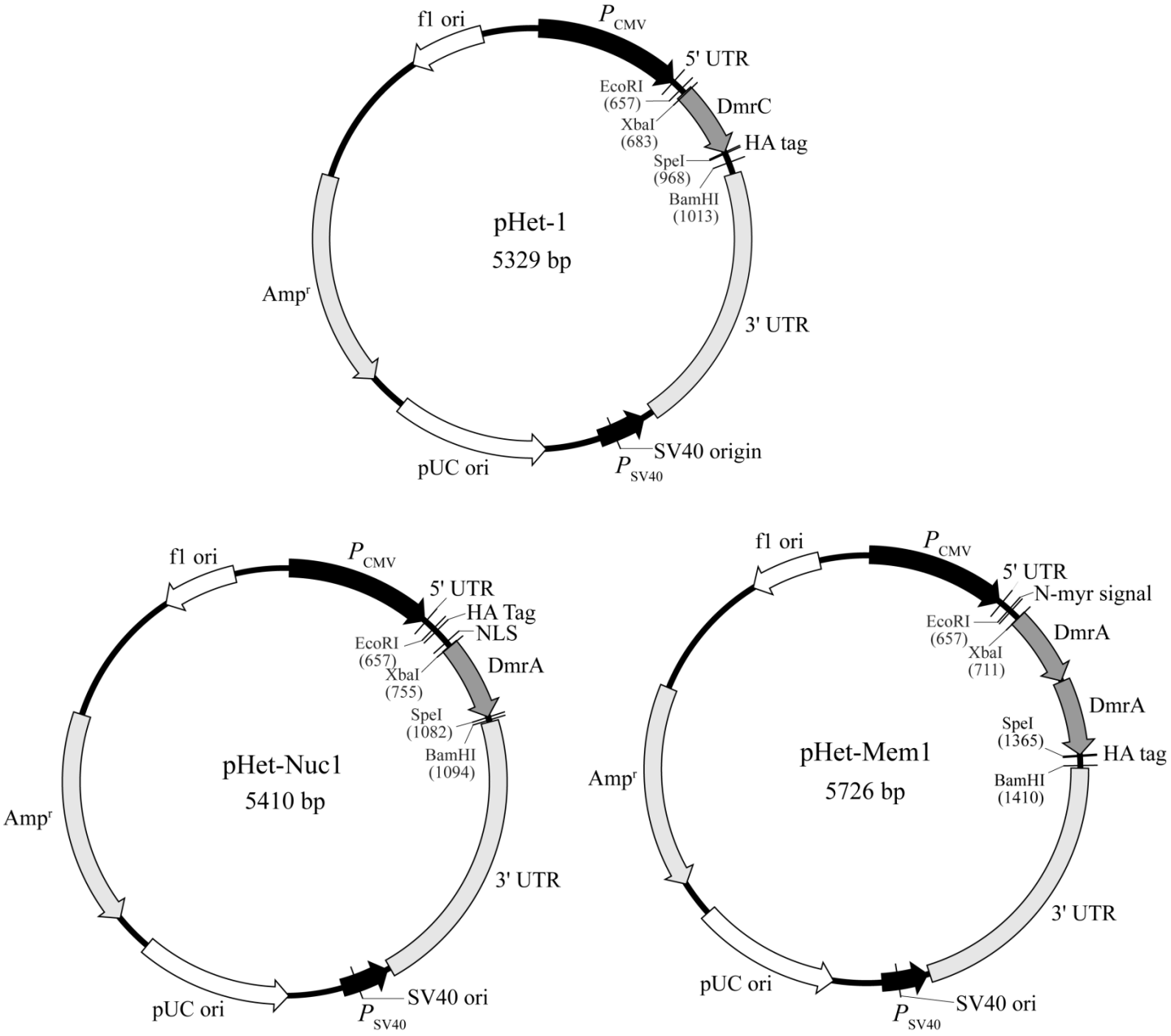

Figure 1. pHet-1, pHet-Nuc1 and pHet-Mem1 Vector Maps. **Cloning Strategy: For prHet-1**, Use the EcoRI and XbaI sites to clone your gene of interest upstream of the dimerization domain, and the SpeI and BamHI sites to clone your gene of interest downstream of the dimerization domain. **For pHet-Nuc1 and pHet-Mem1**, use the XbaI site to clone your gene of interest upstream of the dimerization domain(s), and the SpeI/BamHI sites to clone your gene of interest downstream of the dimerization domain(s). See the iDimerize Inducible Heterodimer System User Manual (PT5179-1) for more information on how to clone your gene of interest. **NOTE:** In pHet-Mem1, the two DmrA-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 two proteins of interest to be tagged with dimerization domains and expressed in mammalian cells. Heterodimerization of the tagged proteins can be induced on demand in live cells when a membrane permeant compound, the A/C Heterodimerizer, is added to the tissue culture medium (1, 2).

To induce the heterodimerization of two proteins of interest, one protein must be expressed as a fusion with DmrA, and the second protein must be expressed as a fusion with DmrC. In the absence of the A/C Heterodimerizer compound, the two proteins of interest will not dimerize. However, when the A/C Heterodimerizer is added to the cell medium, DmrA and DmrC will bind to the compound at different sites, forming a heterodimer. A protein fused to two copies of DmrA can form a potential trimer with a DmrC tagged protein of interest. For proteins that are activated by dimerization, the dimerization of the fusion proteins results in activation. Each DmrA tag also contains a localization signal that determines where the ligand-induced interaction will occur.

The iDimerize Inducible Heterodimer Vector Set 1 contains three mammalian expression vectors that each encodes a different set of dimerization tags:

- **pHet-1** constitutively expresses a protein of interest fused to the dimerization domain DmrC.
- **pHet-Nuc1** constitutively expresses a protein of interest fused to one copy of the dimerization domain DmrA and a nuclear localization sequence (NLS). As a result of the NLS tag, a protein of interest expressed by this vector will localize to the nucleus.
- **pHet-Mem1** constitutively expresses a protein of interest fused to two copies of DmrA and a myristoylation signal sequence (N-myr signal). As a result of the N-myr signal sequence, a protein of interest expressed by this vector will localize to the inner leaflet of the plasma membrane (3).
- The Dmr domains encoded by all three 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).

All three 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

pHet-1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- DmrC (dimerization domain C): 676–966
- HA Tag (hemagglutinin epitope tag): 973–999
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1018–2179
- P_{SV40} (SV40 promoter): 2188–2383 (complementary)
- SV40 origin of replication: 2266–2343 (complementary)
- pUC origin of replication: 2605–3224 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 3379–4251 (complementary)
- f1 origin of replication: 4820–5126 (complementary)

pHet-Nuc1 Vector

- P_{CMV} (human cytomegalovirus promoter): 19–613
- 5′ UTR (HSV TK 5′ untranslated region): 615–655
- HA Tag (hemagglutinin epitope tag): 673–711
- NLS (nuclear localization signal): 733–753
- DmrA (dimerization domain A): 754–1080
- 3′ UTR (rabbit beta-globin 3′ untranslated region, includes polyA signal): 1099–2260
- P_{SV40} (SV40 promoter): 2269–2464 (complementary)
- SV40 origin of replication: 2347–2424 (complementary)
- pUC origin of replication: 2686–3305 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 3460–4332 (complementary)
- f1 origin of replication: 4901–5207 (complementary)

pHet-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
- DmrA (dimerization domain A): 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 Heterodimer System User Manual (PT5179-1) for information on how to clone your gene of interest into your vector of choice. Both DmrA- and DmrC-tagged proteins must be expressed in the same cell. 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 A/C Heterodimerizer, the proteins tagged with DmrA and DmrC will not interact with each other. The addition of the A/C Heterodimerizer to the cell medium induces the interaction between the two tagged proteins. The localization signal on the DmrA tag determines where the ligand-induced interaction will occur.

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. Graef, I. A. *et al.* (1997) *Embo J.* **16**(18):5618–56328.
2. Castellano, F. *et al.* (1999) *Curr Biol.* **9**(7):351–360.
3. 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
pHet-1	SpeI	5.3 kb
	EcoRI & BamHI	0.4 & 5.0 kb
pHet-Nuc1	SpeI	5.4 kb
	EcoRI & BamHI	0.4 & 5.0 kb
pHet-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

iDimerize Inducible Heterodimer Vector Set 1

CATALOG NO.
635063

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