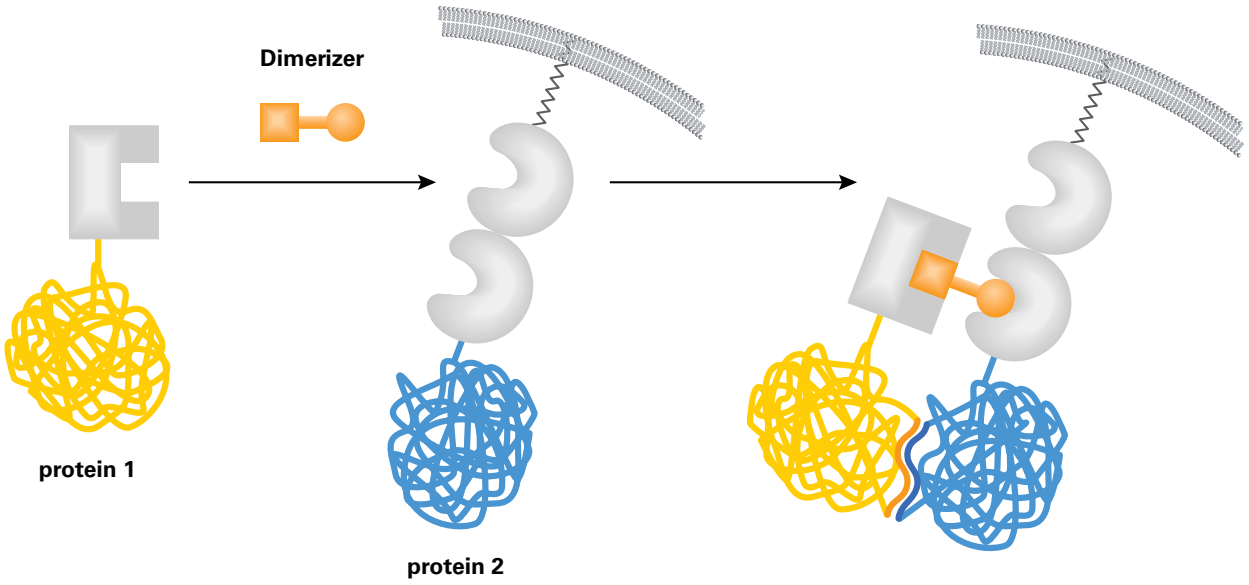
INDUCIBLE SYSTEMS

Switch On Protein-Protein Interactions

Rapid and specific control of signal transduction pathways, protein activity, protein localization, transcription, and more…



Clontech

Clontech is the exclusive provider of iDimerize products—inducible dimerization systems and cell-permeant ligands which were previously provided by ARIAD Pharmaceuticals, Inc. under the brand name ARGENT. This technology has been used by over 2,000 investigators in 35 countries, with over 400 scientific publications to date.

Why Manipulate Protein-Protein Interactions?

- Many critical processes in the cell require protein interactions. In fact, the majority of human proteins can form oligomers—including most cell surface receptors and >70% of human enzymes.
- Inducible protein-protein interaction technology can be applied to any biological process that can be manipulated by influencing the interactions/localization of a protein.
- iDimerize gives you small molecule control of the pathway involvement, activity, or location of your protein of interest.
- With iDimerize, proteins interact specifically and rapidly.

Table I: Just *Some* of the Published Processes Controllable by iDimerize Technology

Cell signaling	Gene transcription
Apoptosis	Enzyme activation
Protein secretion	Protein relocalization
Pathway activation	Protein synthesis
Cell adhesion	Cell rolling
Protein splicing	RNA splicing
Glycosylation	DNA looping
Neurite growth	Transformation
Amyloid fibril formation	Substitute your research interest here...

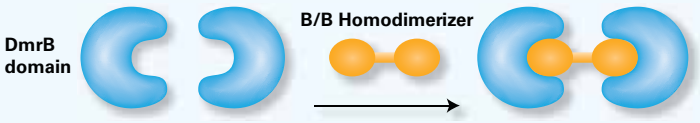
How Does iDimerize Work?

A chemical inducer of dimerization, or "dimerizer", is a cell-permeant organic small molecule with two separate motifs that each bind with high affinity to a specific protein module (Dmr domain) fused onto the protein(s) of interest. Addition of the dimerizer brings the chimeric protein subunits into very close proximity to each other, mimicking the activation of the cellular event that dimerization of the protein of interest controls (1, 2). Conversely, a reverse dimerizer ligand will bind to and dissociate a protein that aggregates in its absence.

Inducible Homodimerization

Induced self-association of two copies of the same protein

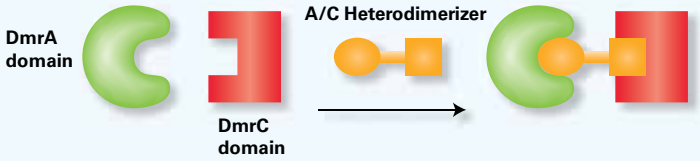
- **Use for *in vitro* and *in vivo* studies, to control a wide variety of cellular processes, including proliferation, differentiation, adhesion, transformation, and apoptosis**



Inducible Heterodimerization

Induced association of two different proteins

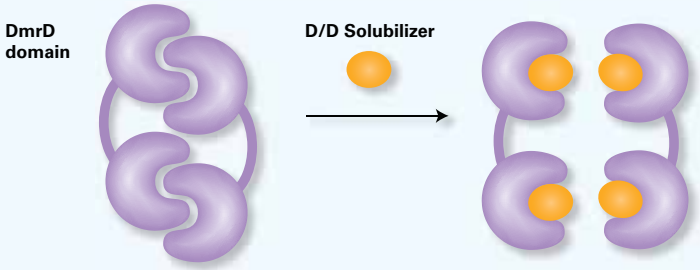
- **Use for *in vitro* and *in vivo* studies, to create conditional alleles of receptors, signaling molecules, or any other protein normally regulated by interactions between two different proteins**



Inducible Reverse Dimerization

Induced dissociation (solubilization or deaggregation) of proteins

- **Use for *in vitro* and *in vivo* studies, to control intracellular location and to induce regulated secretion**



Small Molecule Control of Signal Transduction Pathways

Many signaling cascades are activated almost exclusively by the interactions of signaling proteins. Cell surface receptor proteins cluster in response to extracellular factors, which leads to the recruitment and activation of intracellular signaling proteins. This ultimately leads to transcription activation, effector protein production, and activation or secretion. Any step of this signaling pathway can be brought under dimerizer control by fusing the proteins involved to domains recognized by the respective dimerizer ligand.

The **iDimerize Inducible Homodimer System** (Cat. No. 635068) uses the B/B Homodimerizer ligand, which incorporates two identical binding motifs, to induce self-association of a single signaling domain or other protein of interest. The **iDimerize Inducible Heterodimer System** (Cat. No. 635067) uses the A/C Heterodimerizer ligand, which contains two different binding motifs, to allow the dimerization of two different proteins of interest, each of which is fused to a different dimerization domain recognized by the heterodimerizer. Lentiviral formats (Lenti-X™ Systems) are also available.

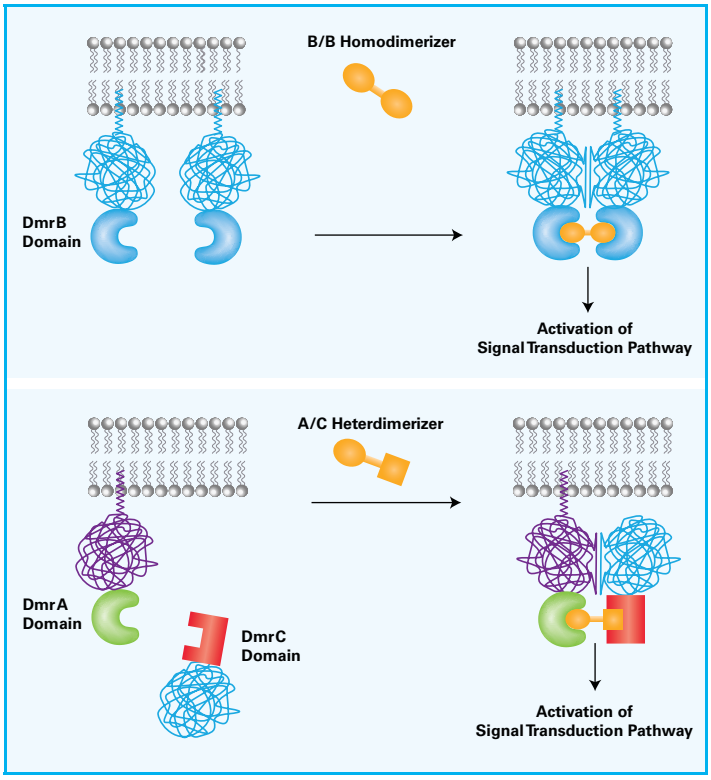


Table II: Types of Signaling Proteins Activated by iDimerize Technology:
Receptor and non-receptor tyrosine kinases
Receptor and non-receptor serine/threonine kinases
Non-kinase receptors
Signaling proteases
Adaptor proteins

Example: Inducing a Programmed Cell Death Pathway (Inducible Apoptosis)

The Fas receptor (FasR) is a transmembrane protein located on the surface of cells that activates programmed cell death (apoptosis) when induced to trimerize by the fas ligand (FasL) located on the surface of adjacent cells (e.g., cytotoxic T cells). FasL/FasR binding plays an important role in the regulation of the immune system and cancer progression. This apoptotic signaling cascade can be mimicked using iDimerize (Figure 1). An *in vivo* model (the MaFIA mouse; 3) utilizes the Fas receptor to systematically and reversibly eliminate macrophages from transgenic mice.

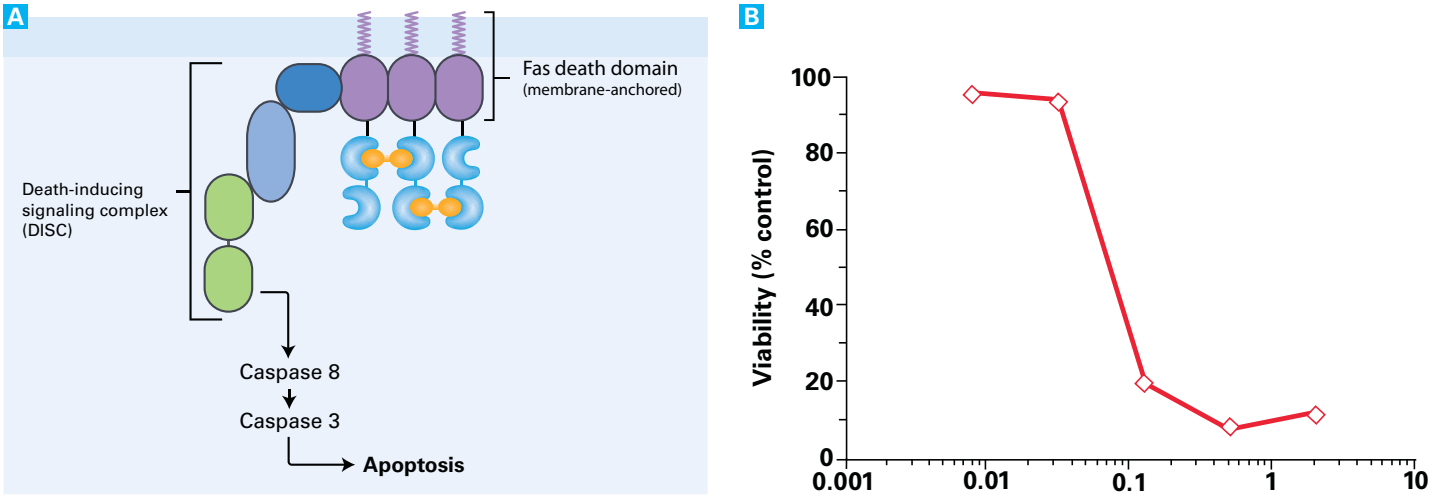


Figure 1. Fas-induced apoptosis *in vitro*. The fas receptor (FasR), once trimerized, activates an apoptosis pathway. Programmed cell death can be mimicked at will by transfecting cells with a construct encoding the Fas-DmrB fusion protein and treating overnight with B/B Homodimerizer to induce trimerization (**Panel A**). Less than 1 nM B/B Homodimerizer was sufficient to induce maximal cell death in these cells (**Panel B**; data kindly provided by ARIAD Pharmaceuticals, Inc.).

Inducible Gene Expression

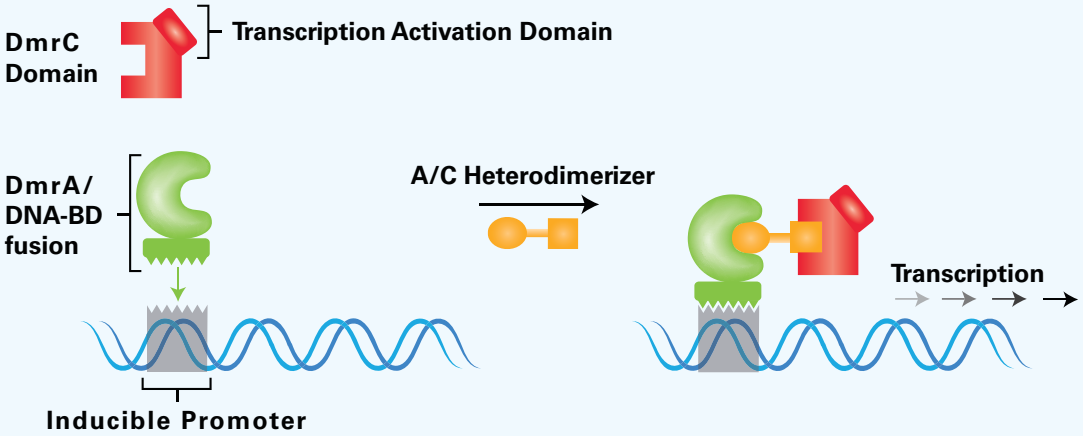
The **iDimerize Inducible Expression System** (Cat. No. 635065), an application kit using heterodimerization technology, can be used to control transcription activation of target genes.

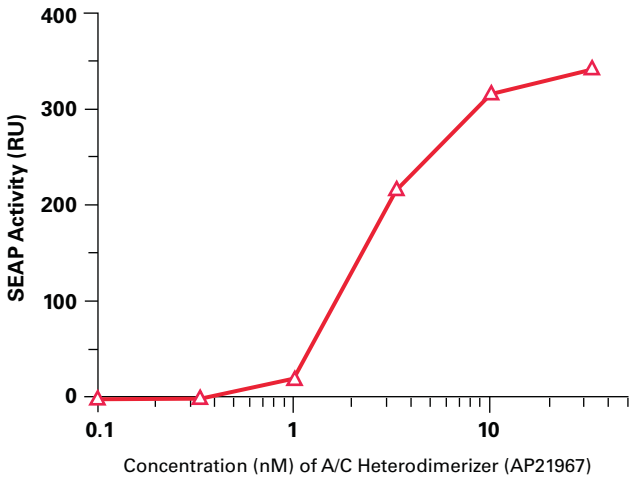
Regulated gene expression using the iDimerize Inducible Expression System.

Clone your gene of interest downstream of the ZHFD1 inducible promoter (P_{ZI-1}). The DNA binding component (DmrA/DNA-BD fusion; green) recognizes and binds sequences within the promoter. However, activation of transcription only occurs when the DmrA/DNA-BD dimerizes with the transcription activation component (DmrC-AD fusion; red) at the promoter, mediated by their mutual affinity for the A/C Heterodimerizer.

The design utilizes three human-based elements:

- Transcription activation component: a single DmrC domain, fused to a transcription activation domain (AD) derived from the p65 subunit of NFkappaB
- DNA binding component: a triplet of DmrA domains, fused to a composite DNA binding domain (BD) called ZFHD1, which consists of two zinc finger domains from Zif268 joined to a homeodomain from Oct-1
- Inducible promoter component (P_{ZI-1}): ZFHD1 binds with high affinity and specificity to 12 repeats of a unique composite ZHFD1 DNA binding sequence, but not to Zif268 or Oct-1 binding sites (4). The binding sites are placed downsteam of a minimal promoter derived from P_{IL2}.





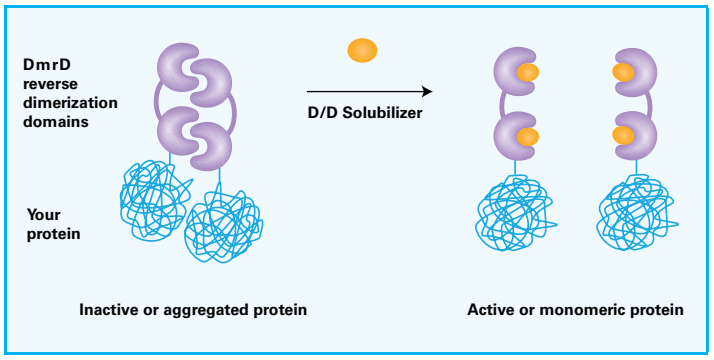
Data kindly supplied by ARIAD Pharmaceuticals Inc.

Figure 2. Dose-dependent control of gene expression with the iDimerize Inducible Expression System. HT1080 cells were stably transfected with the secreted alkaline phosphatase (SEAP) reporter gene and the DmrC-AD/DmrA/DNA-BD constructs, and treated with increasing concentrations of A/C Heterodimerizer. In the absence of A/C Heterodimerizer, target gene expression was undetectable. Half-maximal induction occurred at 2 nM A/C Heterodimerizer.

Temporal Control of Protein Secretion

In the **iDimerize Reverse Dimerization System** (Cat. No. 635066) aggregation is the resting state, and the D/D Solubilizer breaks up protein-protein interactions. This version of the technology can be used for rapid, reversible changes in the subcellular location, aggregation state and/or biological activity of engineered proteins.

An innovative application of this technology is inducible protein secretion, described in Figure 3. The D/D Solubilizer ligand can be added to induce protein secretion from cells in a matter of 15 minutes (Figure 4). This method has been used to induce rapid, transient, and tightly-regulated secretion of insulin in a mouse model for diabetes. Induced release of insulin reversed hyperglycemia (5).



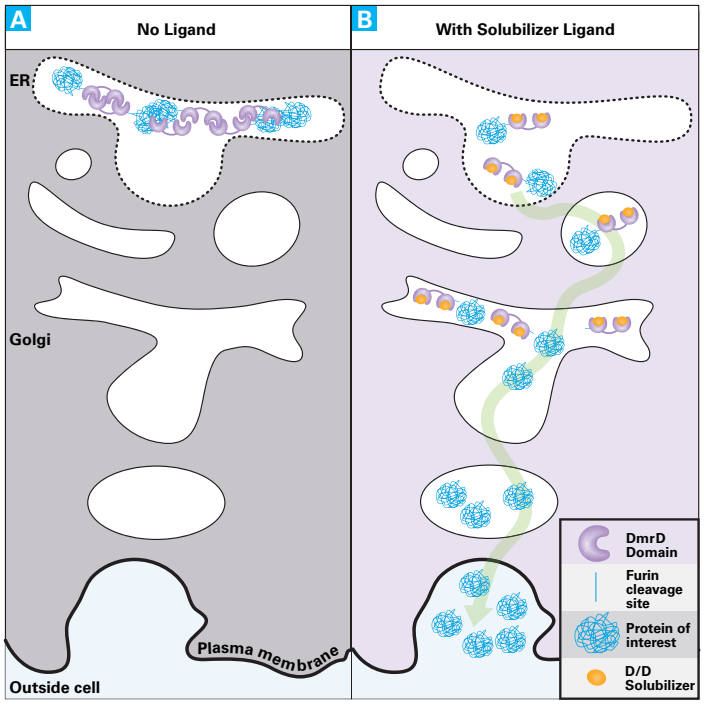


Figure 3. The iDimerize Reverse Dimerization System enables dose-dependent control of protein secretion. Fusion proteins containing DmrD domains localize to the endoplasmic reticulum as aggregates (**Panel A**). When the D/D Solubilizer is added, it dissolves the aggregates and allows the protein to be exported through the secretory apparatus (**Panel B**).

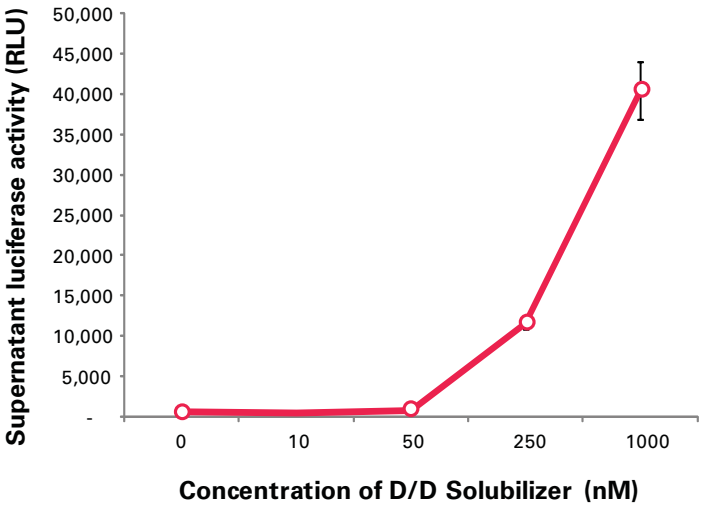


Figure 4. Secretion of DmrD-tagged luciferase after addition of D/D Solubilizer. 7 hr after transfection with a DmrD-tagged *Metridia luciferase* construct, cells were split into wells of a 6-well plate. The medium was removed and fresh medium was added containing increasing concentrations of D/D Solubilizer. 18 hr later, the media was collected and analyzed using Clontech's Ready-To-Glow™ Secreted Luciferase Reporter System (Cat. No. 631731).

Product	Size	Cat. No.
Plasmid Systems (include 500 µl aliquot of dimerizer ligand)		
iDimerize Inducible Homodimer System	each	635068
iDimerize Inducible Heterodimer System	each	635067
iDimerize Reverse Dimerization System	each	635066
iDimerize Inducible Expression System	each	635065
Lentiviral Systems (include 500 µl aliquot of dimerizer ligand)		
Lenti-X iDimerize Inducible Homodimer System	each	635072
Lenti-X iDimerize Inducible Heterodimer System	each	635074
Lenti-X iDimerize Reverse Dimerization System	each	635076
Dimerizer Ligands (*in vitro* format—supplied at 0.5 mM in ethanol)		
B/B Homodimerizer	500 µl	635060
B/B Homodimerizer	5 x 500 µl	635059
A/C Heterodimerizer	500 µl	635057
A/C Heterodimerizer	5 x 500 µl	635056
D/D Solubilizer	500 µl	635054
D/D Solubilizer	5 x 500 µl	635053
Dimerizer Ligands (*in vivo* format—supplied dry)		
B/B Homodimerizer	25 mg	635069
B/B Homodimerizer	5 mg	635058
A/C Heterodimerizer	5 mg	635055
D/D Solubilizer	5 mg	*

References

1. Clackson, T. *et al.* (1998) Redesigning an FKBP-ligand interface to generate chemical dimerizers with novel specificity. *Proc. Natl. Acad. Sci. USA* **95**(18): 10437–10442.
2. Clackson, T. (2007) In *Chemical Biology*: From Small Molecules to Systems Biology and Drug Design, Eds. Schreiber, S. L. *et al.* (Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany), pp. 227–249.
3. Burnett, S. H. *et al.* (2004) Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J. Leukoc. Biol.* **75**(4):612–623.
4. Pomerantz, J. L. *et al.* (1995) Structure-based design of transcription factors. *Science* **267**(5194):93–96.
5. Rivera, V. M. *et al.* (2000) Regulation of protein secretion through controlled aggregation in the endoplasmic reticulum. *Science* **287**(5454):826–830.