Quick & Reversible Control of Your Protein of Interest

Manipulate protein quantity in cells and organisms with fast kinetics, using the ProteoTuner™ Systems

- **Quickly stabilize or destabilize your protein of interest**
- **Gain firsthand information about protein function**
- **Tunable & repeatable for precise control of protein levels**

Analyzing protein function is a key focus in discovery-based cell biology research. Clontech's revolutionary new **ProteoTuner Systems,** based on a technology developed by Dr. Thomas Wandless and colleagues (1), allow you to directly investigate the function of a specific protein of interest—by manipulating the level of the protein itself. This technology has already been successfully used in a variety of applications and organisms, resulting in several publications in outstanding peer-reviewed journals (1–7).

The ProteoTuner Systems are based on a 12 kDa mutant of the FKBP protein (the destabilization domain, or DD) that can be expressed as a tag fused to your protein of interest. The DD fusion protein is reversibly protected from proteasomal degradation in the presence of the small (750 Da) membrane-permeable ligand, **Shield1** (Figure 1).

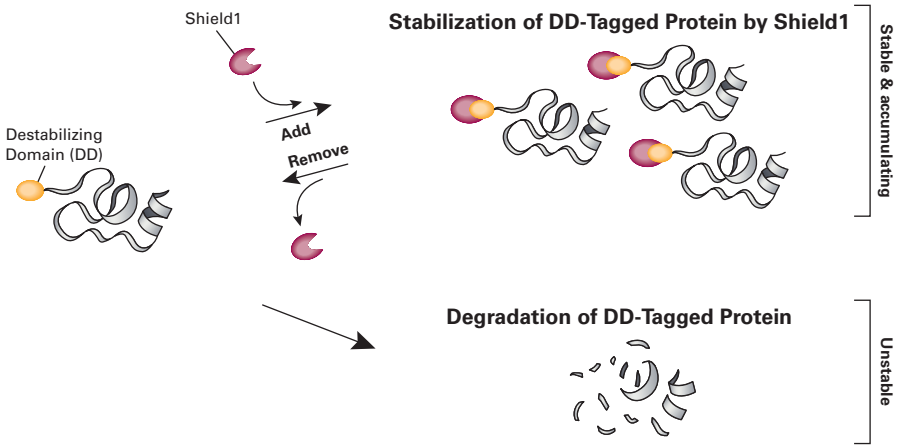

Figure 1. Ligand-dependent, targeted, and reversible protein stabilization. A small destabilization domain (DD) is fused to a target protein of interest. The small membrane-permeable ligand Shield1 binds to the DD and protects it from proteasomal degradation. Removal of Shield1, however, causes rapid degradation of the entire fusion protein. The default pathway for the ProteoTuner Systems is degradation of the fusion protein, unless Shield1 is present.

Fast, Focused Results

Quickly changing the amount of your protein of interest within a cell enables you to gain valuable information about its function. Unlike other systems which regulate the amount of a protein indirectly (at the transcriptional level), this system targets the protein of interest itself, guaranteeing a much quicker response than other methods. It has been shown that a DD fusion protein can accumulate to detectable levels in just 15–30 minutes after adding the stabilizing ligand Shield1 (1).

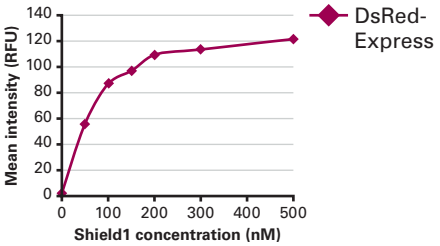

Figure 2. The fluorescence intensity of DD-DsRed-Express is directly related to the concentration of the stabilizing ligand Shield1. Cells were infected with pRetroX-PTuner DsRed-Express and treated with different concentrations of Shield1. The amount of DD-tagged DsRed-Express stabilized by different concentrations of Shield1 was detected by fluorescence intensity 18 hr later, using a BD FACSCalibur™ flow cytometer. RFU = relative fluorescence units.

Adjustable Protein Stabilization

In the presence of Shield1, the DD-tagged protein of interest is stabilized and accumulates inside the cell. Conversely, in its absence, the DD-tagged protein is degraded very rapidly by proteasomes. Thus, it is possible to "tune" the amount of stabilized, DD-tagged protein present in the cell by titrating the amount of Shield1 in the culture medium (Figures 2–3).

The degree of stabilization increases as the Shield1 concentration increases within the range of ~50–1,000 nM.

This was demonstrated by cloning DsRed-Express into the **pRetroX-PTuner Vector** (sold as part of Cat. No. 632171) in-frame with the 5' sequence encoding the DD. Infected cells were treated with different concentrations of Shield1, and the fluorescence intensity of treated cells was analyzed using flow cytometry. Higher fluorescence intensities were observed in samples treated with higher concentrations of Shield1, due to greater amounts of stabilized DD-DsRed-Express protein present in the cells (Figure 2).

These results were confirmed by Western blot, using the **Living Colors® DsRed Polyclonal Antibody** (Cat. No. 632496). DD-DsRed-Express can be stabilized to levels comparable with levels of the untagged protein in cells stably expressing untagged DsRed-Express from the CMV promoter (Figure 3). At lower concentrations of Shield1, there is a direct relationship between the concentration of Shield1 and the amount of destabilized DsRed-Express protein detected by flow cytometry or in the cell lysate (Figures 2 and 3, respectively).

Quick & Reversible Control of Your Protein...continued

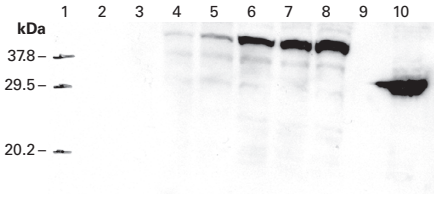

Figure 3. DD-DsRed-Express fluorescence is directly related to the concentration of the stabilizing ligand Shield1. In order to visualize the amount of DD-DsRed-Express expressed in a cell, cells were infected with pRetroX-PTuner DsRed-Express IRES ZsGreen1 and treated with different concentrations of Shield1. The amount of DD-tagged DsRed-Express stabilized by different concentrations of Shield1 was detected via Western blot using the Living Colors DsRed Polyclonal Antibody. Lane 1: molecular weight marker. Lane 2: 1X loading buffer. Lane 3: untreated HeLa cells (no virus, no Shield1). Lane 4: HeLa cells infected with the DD-DsRed Express construct; no Shield1. Lanes 5–8: HeLa cells infected with the DD-DsRed-Express construct and treated with 50, 250, 500, and 1,000 nM Shield1 respectively. Lane 9: 1X loading buffer. Lane 10: HEK 293 DsRed-Express stable cell line.

Reversible & Repeatable

The ProteoTuner method is not restricted to protein *stabilization*—it can also be used to *destabilize* your protein of interest by withdrawing Shield1 from cell cultures that previously contained Shield1. This makes it possible to repeatedly stabilize and destabilize your protein using the same set of cells (1; see pages 9–10).

The ability to quickly and directly control protein levels with the ProteoTuner Systems provides you with a novel tool for studying transient effects that might otherwise be masked, by directly and specifically "tuning" the level of a protein of interest in the cell. Four systems are available: with either plasmid or retroviral vectors, and with or without a Living Colors Fluorescent Protein marker for transfection efficiency (Table 1). Shield1 is available as part of each system, as well as separately.

Table I: Four Single-Vector ProteoTuner Systems Now Available

System	Vector Type	Antibiotic Resistance[1]	Fluorescent Protein
ProteoTuner System	Plasmid	Kanamycin/G418	None
ProteoTuner IRES2 System	Plasmid	Kanamycin/G418	AcGFP1
Retro-X™ ProteoTuner System	Retroviral	Ampicillin/Puromycin	None
Retro-X ProteoTuner IRES System	Retroviral	Ampicillin/None	ZsGreen1

1 Bacterial/eukaryotic

Product	Size	Cat. No.
ProteoTuner System	each	632172
ProteoTuner IRES2 System	each	632168
Retro-X ProteoTuner System	each	632171
Retro-X ProteoTuner IRES System	each	632167
Shield1	60 µl	631037
	200 µl	631038
Living Colors DsRed Polyclonal Antibody	100 µl	632496

ProteoTuner™ System Components

- pPTuner Vector
- Shield1

ProteoTuner™ IRES2 System Components

- pPTuner IRES2 Vector
- Shield1

Retro-X™ ProteoTuner™ System Components

- pRetroX-PTuner Vector
- Shield1

Retro-X™ ProteoTuner™ IRES System Components

- pRetroX-PTuner IRES Vector
- Shield1

Related Products

- Tet-On® Advanced Inducible Gene Expression System (Cat. No. 630930)
- Tet-Off® Advanced Inducible Gene Expression System (Cat. No. 630934)
- Knockout™ Single Vector Inducible RNAi System (Cat. No. 630933)

Notice to Purchaser

Please see the CMV Sequence, Living Colors® Fluorescent Protein Products, and ProteoTuner™ Protein Stabilization/Destabilization Products licensing statements on page 33.

References

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2. Banaszynski, L. A. and Wandless, T. J. (2006) *Chem. Biol.* **13**(1):11–21.
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