Cycle Protein Levels—With Lentiviral Vectors

Control the level of your protein of interest with the Lenti-X™ ProteoTuner™ Systems

- Protein stabilization on demand
- Quickly tune the level of your protein of interest
- Delivery to any cell type

Direct control over the amount of a specific protein is a powerful tool to discover its function in the cell. This type of direct control is now available in the **ProteoTuner Systems.** Each system is based on a 12 kDa destabilization domain (DD) that can be expressed as a tag, fused to your protein of interest, to induce proteasomal degradation of the fusion protein. However, when the small (750 Da) membrane-permeant ligand, **Shield1,** is added to your cells, the DDtagged fusion protein is reversibly protected from proteasomal degradation, causing it to accumulate quickly (Figure 1; 1). With the Proteo Tuner systems, your protein level can be cycled up and down rapidly by the sequential addition and removal of Shield1 (2).

Delivery to Any Cell Type

The Lenti-X ProteoTuner Systems, combined with the Lenti-X HT Packaging System, facilitate efficient delivery of ProteoTuner technology into any cell type. The Lenti-X Systems provide the broad cellular tropism of VSV-G pseudotyped lentivirus, extremely high viral titers, and excellent transgene expression (3). The pLVX-PTuner vectors have been shown to produce high viral titers (e.g., 2.4 x 10⁷ ifu/ml) similar to those of our other Lenti-X vectors (data not shown; 3).



Figure 1. Ligand-dependent, targeted and reversible protein stabilization. A small destabilization domain (DD; gold) is fused to a target protein of interest. The small membrane-permeant ligand Shield1 (red) binds to the DD and protects the fusion protein from proteasomal degradation, allowing it to accumulate rapidly in the cell. Conversely, removal of Shield1 causes rapid degradation of the fusion protein. The default pathway for the ProteoTuner systems is degradation of the fusion protein, unless Shield1 is present.



Figure 2. pLVX-PTuner Vectors for controllable protein cycling. The pLVX-PTuner vector offers puromycin selection capability and is included with the Lenti-X ProteoTuner System. The pLVX-PTuner Green vector provides fluorescent protein coexpression (ZsGreen1) and is included with the Lenti-X ProteoTuner Green System.

Product	Size	Cat. No.	
ProteoTuner	System		
	each	632172	
ProteoTuner	IRES2 System		
	each	632168	
Retro-X Prot	eoTuner System		
	each	632171	
Retro-X Prot	eoTuner IRES Sy	stem	
	each	632167	
Lenti-X Prote	oTuner System		NE
	each	632173	
Lenti-X Prote	oTuner Green S	/stem	NE
	each	632175	
Shield1*			NE
	60 µl	631037	
	200 µl	631038	
	500 µl	632189	
Lenti-X HT P	ackaging System	1	
	20 rxn	632160	
	40 rxn	632161	

* The number of reactions depends on the concentration of Shield1 used. At the maximum suggested concentration (1,000 nM) 60 µl = 30-plus reactions in a six-well plate.

ProteoTuner[™] System Components

- Each system contains a PTuner vector (pPTuner, pPTuner IRES2, pRetroX-PTuner, pRetroX-PTuner IRES, pLVX-PTuner, or pLVX-PTuner Green)
- Shield1

Notice to Purchaser

Please see the BGH Poly A, CMV Sequence, cPPT Element, Lentiviral Expression Products, Living Colors® Fluorescent Protein Products, ProteoTuner™ Protein Stabilization/Destabilization Products, Tet-Based Expression Products, VSV-G Technology and WPRE Technology licensing statements on page 25.

Once your gene of interest is cloned into the included pLVX-PTuner vector (Figure 2), the DD-tagged fusion protein of interest can be expressed in almost any mammalian cell type, including primary cultures, nondividing cells, stem cells, and neurons (see pages 6–7).

References

- Quick & Reversible Control of Your Protein of Interest (April 2008) *Clontechniques* XXIII(2):1–2.
- Banaszynski, L. A. *et al.* (2006) *Cell* 126(5):995–1004.
- High Efficiency Lentiviral Packaging (October 2007) *Clontechniques* XXII(4):1–2.