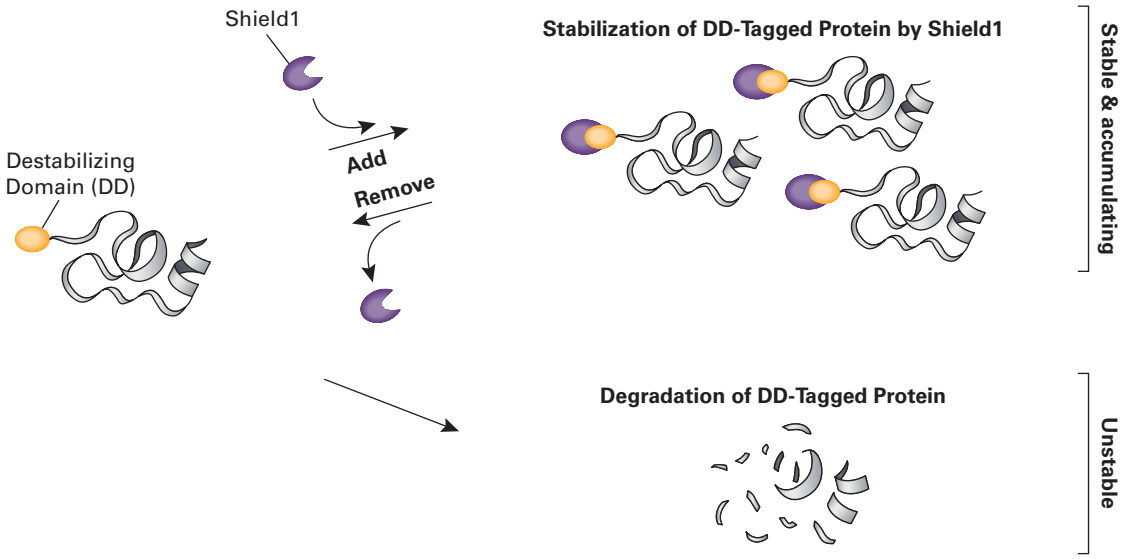
New Solutions for Accurate Protein Function Studies

- **On-demand, reversible, and tunable regulation**
- **Multiple options for tags and delivery**

Rapid & Precise Protein Control with Active On/Off Switching

Analyzing protein function is a key focus in cell biology research. Clontech's innovative ProteoTuner™ systems make it possible to investigate the function of a specific protein of interest directly—by rapidly changing the abundance of the protein itself. These systems utilize ligand-dependent destabilization domains (DDs) and their membrane-permeant ligand Shield1 to reversibly stabilize a DD-tagged protein of interest in a predictable and dose-dependent manner. Rapid kinetics, an active degradation mechanism, and direct control of protein stability enable precise characterization of the fusion protein of interest in live cells. ProteoTuner technology has already been successfully used in a wide variety of applications (Table I).



Ligand-dependent, targeted, and reversible protein stabilization with the ProteoTuner Systems. The default pathway is degradation of the fusion protein, unless Shield1 is present. The same mechanism applies to DD-N and DD-C fusions.

Table I: Selected Applications of ProteoTuner Technology

Application	Reference
Monitoring ion channel composition and function	Schoeber, J. P. H. *et al.* (2009) *Am. J. Physiol. Renal Physiol.* **296,** F204–211.
Manipulating gene repair efficacy of a zinc finger nuclease	Pruett-Miller, S. M. *et al.* (2009) *PLoS Genetics* **5**(2):e1000376.
Characterizing tumor formation in mice	Banaszynski, L. A. *et al.* (2008) *Nat. Med.* **14**(10):1123–1127.
Analyzing essential gene function in apicomplexan parasites	Agop-Neresian, C. *et al.* (2009) *PLoS Pathogens* **5**(1):e1000270. Armstrong, C. M. and Goldberg, D. E. (2007) *Nat. Meth.* **4**(12):1007–1009. Herm-Götz, A. *et al.* (2007) *Nat. Meth.* **4**(12):1003–1005. *Erratum in: Nat. Meth.* (2008) **5**(1):113.
Tracking dynamic cytoskeletal reorganization	On-Demand Protein Stabilization Using the Lenti-X™ ProteoTuner Systems (October 2008) *Clontechniques* **XXIII**(3):2–3.

Create Your Fusion of Choice

All of the ProteoTuner systems are based on a DD which is fused to the target protein of interest. The DD's small, membrane-permeant ligand, Shield1, binds to the DD-tagged protein and protects it from proteasomal degradation, causing rapid accumulation in the cell. Removal of Shield1, however, causes accelerated, dose-dependent degradation of the entire fusion protein (1, 2).

Our latest ProteoTuner Systems include a version of the DD that is optimized for use as a C-terminal tag (DD-C; 3). DD-C exhibits low basal expression and high inducibility (Figure 1), which is comparable to that obtained with N-terminal fusions (2).

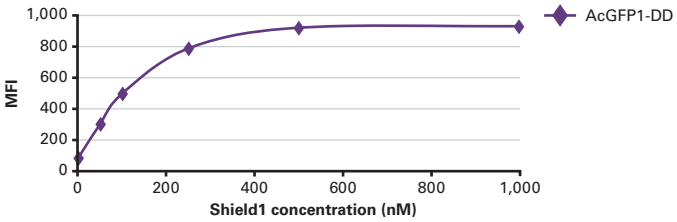


Figure 1. Shield1 regulates DD-C fusion protein levels in a dose-dependent manner. HEK 293 cells expressing AcGFP1-DD were treated with varying concentrations of Shield1. 12 hr later, the amount of AcGFP1 stabilized by different concentrations of Shield1 was detected by fluorescence intensity using flow cytometry. MFI = mean fluorescence intensity.

Easy Antibody Detection

The DD Monoclonal Antibody specifically detects the DD N- and C-terminal tags (Figure 2). It can be used to identify and confirm fusion constructs in cell lysates by Western blot, and for immunocytochemistry. The antibody is highly sensitive: it can detect DD-tagged protein from as few as ~10,000 cells transiently transfected with DD-AcGFP1 (data not shown).

References

1. Banaszynski, L. A. *et al.* (2006) *Cell* **126**(5):995–1004.
2. Quick & Reversible Control of Your Protein of Interest (April 2008) *Clontechniques* **XXIII**(2):1–2.
3. Chu, B. W. *et al.* (2008) *Bioorg. Med. Chem. Lett.* **18**(22):5941–5944.

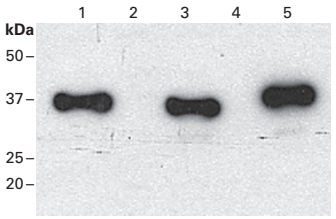


Figure 2. Easy detection of DD fusions with the DD Monoclonal Antibody. Cell lysates from HeLa cells transiently expressing either DD-AcGFP1 or AcGFP1-DD, and HEK 293 cells stably expressing DD-AcGFP1, were analyzed by Western blot using the DD Monoclonal Antibody at a 1:500 dilution. Lane 1: HeLa cells transfected with pDD-AcGFP1 (e.g., DD-N). Lane 2: Negative control (untransfected HeLa cells). Lane 3: HeLa cells transfected with pAcGFP1-DD (e.g., DD-C). Lane 4: Negative control (untransfected HEK 293 cells). Lane 5: HEK 293 cells stably expressing DD-AcGFP1.

Ordering Information

Product	Size	Cat. No.	
ProteoTuner C System	each	631072	**NEW!**
Lenti-X ProteoTuner C System	each	631074	**NEW!**
DD Monoclonal Antibody	50 µl	631073	**NEW!**
ProteoTuner System	each	632172	
ProteoTuner-IRES2 System	each	632168	
Retro-X ProteoTuner System	each	632171	
Retro-X ProteoTuner IRES System	each	632167	
Lenti-X ProteoTuner System	each	632173	
Lenti-X ProteoTuner Green System	each	632175	
ProteoTuner Quantitation System	each	632196	
Shield1*	60 µl 200 µl 500 µl	631037 631038 632189	
Shield1 *in vivo*	each	632188	

* The number of reactions depends on the concentration of Shield1 used. At the maximum suggested concentration (1,000 nM), 60 µl = 30-plus reactions and 200 µl = 1,000-plus reactions in a six-well plate.

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Please see the CMV Sequence, cPPT Element, Lentiviral Expression Products, Living Colors® Fluorescent Protein Products, ProteoTuner™ Protein Stabilization/Destabilization Products, Retroviral Vectors, and WPRE Technology licensing statements at **www.clontech.com/licensing**