I. Introduction

This protocol is provided for use with the **Lenti-X Actin Dynamics Monitoring Kit** (Cat. No. 631076). Use this kit to monitor the highly dynamic rearrangement of the actin filament system in live cells. For a general introduction to the theory of the ProteoTuner™ and Lenti-X Lentiviral Expression Systems, please refer to their respective User Manuals (ProteoTuner Plasmid-Based Shield Systems User Manual for the ProteoTuner Systems and PT3983-1 for the Lenti-X Systems). Both manuals can be found at **www.clontech.com/manuals**. For the specifics of the Lenti-X Actin Dynamics Monitoring Kit protocol, refer only to this Protocol-at-a Glance.

II. Protocol Overview

After packaging both vectors into lentiviral particles (see PT3983-1), transduce your target cells with LVX-mCherry-Actin and LVX-DD-AcGFP1-Actin. These encode human β-actin fusions to mCherry (red, stable) and to DD-AcGFP1 (green, destabilized) respectively.

Once your cells have been transduced, DD-AcGFP1-Actin is continuously targeted for degradation by the proteasomes, unless the cells are cultured in medium containing the stabilizing ligand Shield1. By contrast, mCherry-Actin, which does not contain the DD (and therefore is not destabilized) is constitutively present in the cell.

Adding the DD's stabilizing ligand Shield1 creates a “pulse-chase”-like environment which allows you to monitor actin filament dynamics in live cells by visualizing the integration of newly synthesized, Shield1-stabilized (green) DD-AcGFP1-Actin into the existing (red) mCherry-Actin actin filament network (Figure 1).

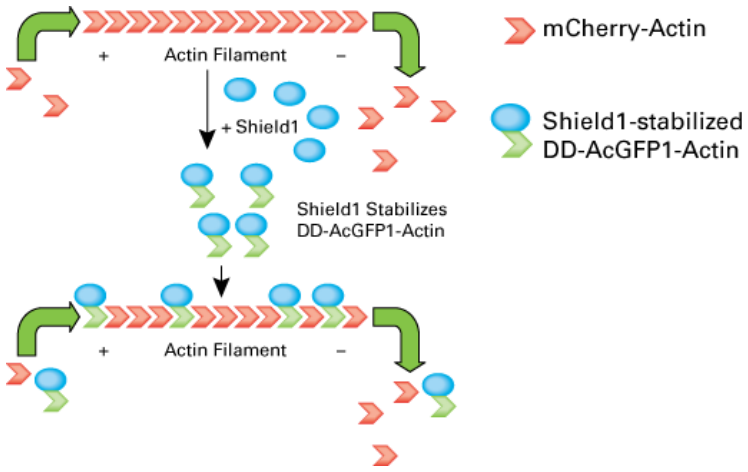

Figure 1. Dynamic polymerization and depolymerization of actin. Self-assembly of actin filaments occurs at the plus end of an existing actin filament, as monomeric actin is incorporated. Conversely, disassembly occurs at the minus end where actin monomers depolymerize from the filament, causing a continuous rearrangement of the actin filament network. In the absence of Shield1, only mCherry-Actin is present to be incorporated into newly forming actin filaments. When Shield1 is added, DD-AcGFP1-Actin is stabilized, and is therefore incorporated together with mCherry-Actin into newly formed actin filaments.

III. Protocol: Monitoring Actin Dynamics

1. Package both vectors into high-titer, replication-incompetent lentiviral particles.

   NOTE: We recommend using the Lenti-X HT Packaging System (Cat. No. 632160) and the Lenti-X 293T Cell Line (Cat. No. 632180).

2. Coinfect your cells of interest with both lentiviral constructs (encoding human β-actin fusions to mCherry (red, stable) and to DD-AcGFP1 (green, destabilized).

3. 12–24 hr posttransfection, split the cells into at least two samples, depending on the number of samples you would like to analyze.

   NOTE: We recommend performing all experiments in duplicate or triplicate.

   - **Adherent cells:** split into at least two parallel cultures. Allow the cells to adhere.

     NOTE: We recommend the use of 6-well plates; however other plate formats can also be used.

   - **Cell suspensions:** distribute suspension evenly into at least two wells.

4. Immediately before beginning your experiment, prepare the following culture medium solutions. The total volume required depends on the number of wells/plates in your experiment.

   - Prewarmed (37°C) culture medium without Shield1.
   - Prewarmed (37°C) culture medium with Shield1 (diluted 1:500 in the culture medium; final concentration 1 µM).

5. Remove the culture medium (from Step 3) and replace with the appropriate culture medium (from Step 4).

   - **Adherent cells:** after the cells have adhered, remove the medium and replace with the appropriate culture medium.
     - **Negative control**: Prewarmed (37°C) culture medium without Shield1.
     - **Experimental sample:** Prewarmed (37°C) culture medium with Shield1 (1 µM).
   - **Cell suspensions:** centrifuge the cells for 5 min at ≤1,000 RPM and remove the medium. Resuspend each pellet in the appropriate culture medium and transfer each suspension back into the appropriate well/plate/flask.
     - **Negative control:** Prewarmed (37°C) culture medium without Shield1.
     - **Experimental sample:** Prewarmed (37°C) culture medium with Shield1 (1 µM).

6. Incubate the cells (adherent or suspended) at 37°C for the time required by your experimental protocol.

7. Observe actin filament behavior via microscopy.

8. Remove the medium containing Shield1.

   - **Adherent cells:** after the cells have adhered, remove the medium containing Shield1. Wash the cells three times with warm culture medium without Shield1. Replace the final wash with fresh culture medium without Shield1.
   - **Cell suspensions:** centrifuge the cells for 5 min at ≤1,000 RPM and remove the medium containing Shield1. Resuspend the pellet in warm culture medium without Shield1 and transfer each suspension back into the appropriate well/plate/flask.

9. Observe actin filament behavior via microscopy using the appropriate red and green filter sets.

   NOTE: To perform a "pulse-chase" style experiment, repeat steps 5–9 can as many times as desired.

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