Protocol-at-a-Glance

DD-Fluorescent Protein Reporter Systems Protocol-at-a-Glance

(PT4088-2)

This protocol is provided for use with the DD-Fluorescent Protein Reporter Systems using vector-based (Cat. Nos. 631079, 631081, 631083, 631085, 631087, 631089, 632190, 632191 & 632192) or lentiviral delivery (Cat. Nos. 631748, 631751 & 631753). For a general introduction to the theory of the ProteoTuner™ and Lenti-X™ Lentiviral Expression Systems, please refer to their respective User Manuals (PT4039-1 for the ProteoTuner Systems and PT5135-1 for the Lenti-X Systems). Both manuals can be found at **www.clontech.com/manuals**. For the specifics of the DD-Fluorescent Protein Reporter Systems protocol, refer only to this Protocol-at-a Glance.

In brief, the experimental protocol is as follows. First, transfect your cells with the pDD-Fluorescent Protein Vector or pLVX-DD-Fluorescent Protein Vector containing the precloned NFκB or CRE promoter, or your promoter of interest. Then, treat the cells under three conditions: without Shield1 or an inducer (the background control), with Shield1 but not the inducer (the negative control), or with Shield1 plus the inducer (the test condition). In the absence of Shield1, the reporter protein is rapidly targeted to and degraded by proteasomes, which minimizes background fluorescence from leaky promoters. When Shield1 is added, the reporter is stabilized; and when the inducer is added together with Shield1, reporter molecules expressed during promoter activation will contribute to the fluorescence signal, providing a high signal-to-noise ratio.

A. Clone your promoter of interest

(Promoterless systems only; Cat. Nos. 631748, 631751, 631753, 632190, 632191 & 632192)

1. Using either standard or In-Fusion™ cloning techniques, insert your promoter of interest into the multiple cloning site (MCS) of the vector included with your system. This promoter will now drive the expression of the fluorescent reporter (DD-AmCyan1, DD-ZsGreen1, or DD-tdTomato).
2. Transfect the resulting construct into your cells of interest by your method of choice.
3. Culture cells for 12–24 hr.

B. Study promoter activity *(all systems)*

1. Split the cells into at least three samples, depending on how many samples you would like to analyze.

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

   - **For adherent cells:** Split your cells into at least three parallel cultures. Allow the cells to adhere.

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

   - **For cell suspensions:** Distribute your cell suspension evenly into at least three wells.
2. Prepare the following culture media solutions. The total volume required depends on the number of wells/plates in your experiment.
   - **Background control** (for basal promoter activity; without Shield1): Prewarmed (37°C) culture medium.
   - **Negative control** (no promoter activation; with Shield1): Dilute the Shield1 stock solution 1:500 in prewarmed (37°C) culture medium, for a final concentration of 1 µM.
   - **Test condition** (with promoter activation *and* Shield1): Dilute the Shield1 stock solution 1:500 in prewarmed (37°C) culture medium, for a final concentration of 1 µM. Add the inducer or other compound of interest to this medium, at the concentration defined by your experimental protocol.

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3. Remove the culture medium (from Step B.1) and replace with the appropriate culture medium (from Step B.2), to determine the background, the negative control, and the effect of the inducer.
   - **For adherent cells:** after the cells have adhered, remove the media and replace with the appropriate culture medium (from Step B.2).
   - **For cell suspensions:** centrifuge the cells for 5 min at ≤1,000 RPM and remove the media. Resuspend each pellet in the appropriate culture medium (from Step B.2) and transfer each suspension back into the appropriate well/plate/flask.
4. Culture the cells (adherent or suspended) at 37°C for the time required by your experimental protocol.
5. Collect the cells for analysis.
   - **For adherent cells:** collect the cells by trypsinizing; then wash and pellet the cells.
   - **For cell suspensions:** wash and collect the cells by gentle centrifugation for 5 min at ≤1,000 RPM.
6. Resuspend the cell pellet in PBS and analyze the fluorescent signal of the cells using flow cytometry. Use the background control from transfected cells cultured in the absence of Shield1 to determine the overall background. Alternatively, the reporter can be detected using a fluorescence plate reader, especially if the experiment was performed in a 96-well format.
7. Calculate the "fold induction" of promoter activity:

$$\frac{\text{Fluorescence intensity of cells treated with Shield1 \& the inducer of interest}}{\text{Fluorescence intensity of cells treated with Shield1 alone}}$$