Destabilized DsRed-Express and HcRed Vectors

Red and far-red fluorescent proteins engineered for rapid turnover

- **Detect transient changes in gene expression**
- **Develop stable cell lines**
- **Monitor multiple events simultaneously—choose from destabilized cyan, green, yellow, and red fluorescent proteins**

Our newest BD Living Colors™ vectors—**pDsRed-Express-DR** and **pHcRed1-DR**—encode destabilized variants of our red and far-red fluorescent proteins DsRed-Express and HcRed1. In contrast to the original proteins, these destabilized variants—**DsRed-Express-DR** and **HcRed1-DR**—have short half-lives, making them well suited for studies that require rapid reporter turnover. These new promoterless vectors can be used to accurately analyze *cis*-acting regulatory elements in studies of gene regulation, and may facilitate routine generation of stable transfectants.

DsRed-Express-DR and HcRed1-DR were constructed by fusing the fluorescent proteins to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (1). This C-terminal region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (1, 2).

Many potential applications

Because of their rapid turnover, DsRed-Express-DR and HcRed1-DR are useful as transcription reporters for measuring both the up- and down-regulation of promoter activity. For example, by placing DsRed-Express-DR or HcRed-DR under the transcriptional control of a *cis*-acting regulatory element, you can develop assays to study the induction and repression of gene expression during signal transduction (Figure 1). Similar constructs could also be designed to explore the programmed changes that occur during embryogenesis and cell differentiation. Such studies have been carried out with destabilized green fluorescent protein (3, 4). And because the fluorescence can be detected without the addition of substrates or cofactors, you can measure the events non-invasively in real time—a real advantage over other transcriptional reporters such as luciferase or β-galactosidase.

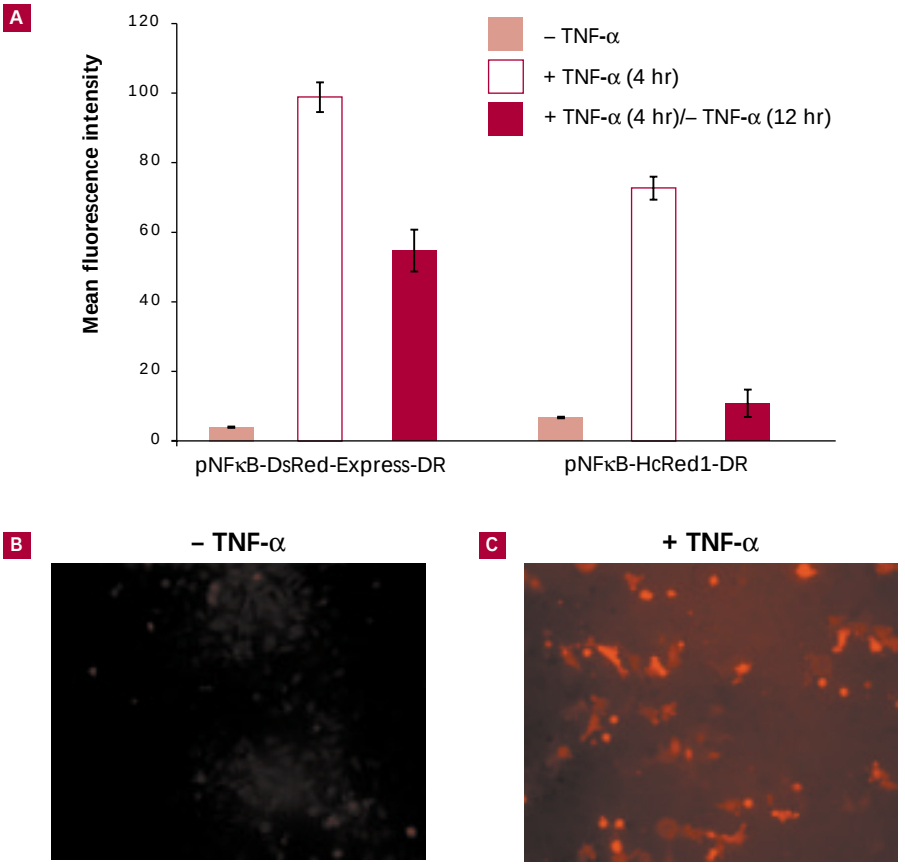

Figure 1. Destabilized red fluorescent proteins measure both the up- and down-regulation of promoter activity. Panel A: To measure the activation of NFκB—a transcription factor known to regulate several genes involved in inflammation, immune response, and apoptosis (7, 8)—the NFκB DNA response element was cloned into the MCS upstream of the fluorescent reporter gene in pDsRed-Express-DR and pHcRed1-DR. The constructs were then transiently transfected into HeLa cells. After overnight incubation, cells were analyzed by flow cytometry using a BD FACSVantage™ SE at three separate times: first to establish the baseline fluorescence; second to measure the fold induction after 4 hours of treatment with 100 ng/ml TNF-α; and third to measure down-regulation 12 hours after withdrawing TNF-α from the culture. In this example, HcRed1-DR was excited with a 568-nm laser line; DsRed-Express with a 488-nm line. **Panels B & C:** Photomicrographs of cells transiently transfected with pNFκB-DsRed-Express-DR before (**Panel B**) and after (**Panel C**) induction. The image was recorded with a Zeiss Axioskop using Chroma Technology Corp filters hq545/50X, 580dcxr, and hq630/60M.

Red fluorescence and rapid turnover heighten the sensitivity of your assays

Destabilized fluorescent proteins have clear advantages over their long-lived counterparts. First, when placed under the control of an inducible promoter, destabilized variants exhibit a higher fold-induction upon activation (Figure 1). That's because the small amount of protein expressed in the uninduced state is rapidly degraded, so the baseline fluorescence in the uninduced state is low—and the lower the baseline, the greater the sensitivity of your assay. Second, with their long-wavelength excitation maxima, destabilized *red* fluorescent proteins such as DsRed-Express-DR and HcRed1-DR eliminate the need for intense, high-energy radiation that may damage cells and tissues, and their long-wavelength emissions stand out sharply against the green autofluorescent background from media, culture ware, and cellular components. (For detailed information about the spectral properties of HcRed1 and DsRed-Express, please see References 5 and 6.)

In some kinetic assays, rapid activation is as important as rapid inactivation. Our data (Figure 1) show that these new

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transcription reporters develop fluorescence soon after induction, as expected from past studies of DsRed-Express and HcRed1, the parent proteins, whose maturation rates compare favorably to that of enhanced green fluorescent protein (EGFP; 5, 6). Similarly, when the inducer is withdrawn, the fluorescence quickly declines due to the rapid turnover of the reporter (Figure 1).

Many destabilized vectors to choose from, cyan, green, yellow—and now red

pDsRed-Express-DR and pHcRed1-DR (Figure 2) join a growing line of BD Living Colors™ cyan, green, and yellow fluorescent vectors. Like our other promoterless vectors, pDsRed-Express-DR and pHcRed1-DR contain an upstream multiple cloning site so that you can join any promoter/enhancer element to the red reporter of your choice: DsRed-Express-DR or HcRed1-DR. The coding sequence for each reporter has been human codon-optimized for efficient translation in mammalian cells. Whether viewed by fluorescence microscopy (Figure 1) or measured by flow cytometry, these reporters emit at distinctive wavelengths that are easily resolved from our cyan, green, and yellow fluorescent variants. So why not take your experiments one step further and combine reporters to monitor two or even three different events simultaneously? The tools are now at hand.

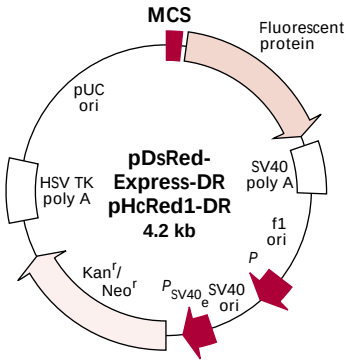

Figure 2. Plasmid map for pHcRed1-DR and pDsRed-Express-DR.

Product	Size	Cat. #
pDsRed-Express-DR Vector	20 µg	6996-1
pHcRed1-DR Vector	20 µg	8114-1

References

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3. Wahlers, A., *et al.* (2001) *Gene Ther.* **8**:477–486.
4. Dorsky, R. I., *et al.* (2002) *Dev. Biol.* **241**:229–237.
5. BD Living Colors HcRed (April 2002) *Clontechniques* **XVII**(2):12–13.
6. BD Living Colors DsRed-Express (July 2002) *Clontechniques* **XVIII**(3):16–17.
7. Jesenberger, V. & Jentsch, S. (2002) *Nature Reviews* **3**:112–121.
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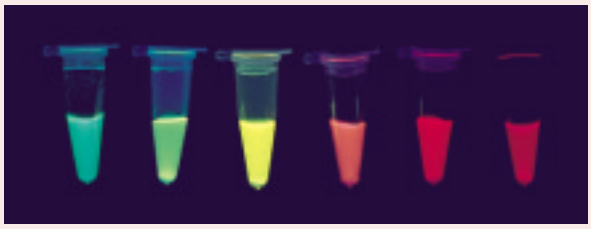

Figure 3. BD Living Colors™ Reef Coral Fluorescent Proteins under UV light. From left to right: AmCyan, ZsGreen, ZsYellow, Dsred, AsRed, and HcRed.

Are you interested in using BD Living Colors Fluorescent Proteins for your internal research? BD Biosciences Clontech offers flexible research and drug discovery licenses that provide access to a complete set of novel Reef Coral Fluorescent Proteins (RCFPs): AmCyan, ZsGreen, ZsYellow, DsRed, AsRed, and HcRed (Figure 3). Six distinct proteins, four brilliant colors—cyan, green, yellow, and three spectrally distinct reds—available exclusively to pharmaceutical and biotech companies through the **BD Living Colors™ Licensing Program**.

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