

# Living Colors™ DsRed2

Improved red fluorescent protein for use in living cells

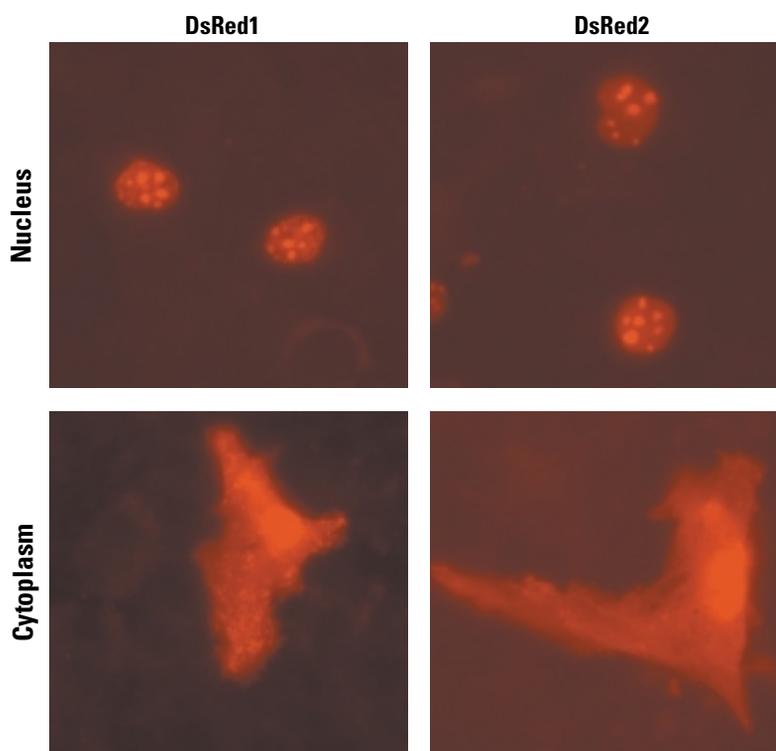
- **Observe fluorescence only 24 hours after transfection**
- **Increased solubility—low tendency to form aggregates in living cells**
- **No cofactors or chemical staining required**

The newest addition to our Living Colors™ reporters—**DsRed2**—expands the utility of our fluorescent proteins. DsRed2 is a variant of our original red fluorescent protein (DsRed1), modified through several point mutations. These mutations improve the solubility of DsRed2 by reducing its tendency to form aggregates and also reduce the time from transfection to detection to only 24 hours. DsRed2 retains the benefits of a red fluorescent protein, such as a high signal-to-noise ratio and distinct spectral properties for use in multicolor labeling experiments (Table 1).

You can use DsRed2 by itself to measure transfection efficiency, to determine intracellular protein localization, or to isolate cells specifically producing your protein of interest. Additionally, DsRed2 can be used in combination with Enhanced Green Fluorescent Protein (EGFP), or any other variant of EGFP, to analyze mixed cell populations by flow cytometry (see page 21), or to monitor gene expression from different promoters. By combining fluorescent reporters, you can also colocalize fusions within a single cell. With the addition of DsRed2 to our other colors of fluorescent proteins, we extend the range of tools available for your research.

## Improved solubility—less protein aggregation

DsRed2 is optimized for high solubility and low aggregation. Use of our original DsRed1 in microscopy studies is limited by its tendency to form aggregates. Figure 1 clearly illustrates visible aggregates in cells expressing DsRed1 targeted to either the cytoplasm or the nucleus. In contrast, no protein aggregates are visible in cells expressing DsRed2 targeted to either the cytoplasm or nucleus. Although DsRed2 probably forms tetramers, the point mutations introduced to generate DsRed2 have drastically reduced the protein's propensity to aggregate nonspecifically, making DsRed2 highly amenable to fluorescence microscopy studies.



**Figure 1. DsRed2 forms no detectable aggregates.** Photomicrographs of DsRed1 and DsRed2 expression in NIH 3T3 cells were taken at 72 hours after transfection. For the nuclear localization study, we transfected NIH 3T3 cells with pDsRed1-Nuc or pDsRed2-Nuc. For the cytoplasmic localization study, we transfected NIH 3T3 cells with pDsRed1-N1 and pDsRed2-N1. While both nuclear and cytoplasmic images of DsRed1-expressing cells showed protein aggregates, images from DsRed2-expressing cells showed no visible protein aggregates.

## Well-tolerated expression in mammalian cells

Comparison of DsRed2 to DsRed1 expression in mammalian cells shows that DsRed2 is better tolerated than DsRed1. We initially transfected 293 cells with either pDsRed1-N1 or pDsRed2-N1. After a two-week selection period, we sorted cells by FACS to remove any nonfluorescent cells. After another four weeks in culture, more than 75 percent of the DsRed2 population fluoresced, while less than 20 percent of the DsRed1 population fluoresced (Figure 2).

## See your results faster

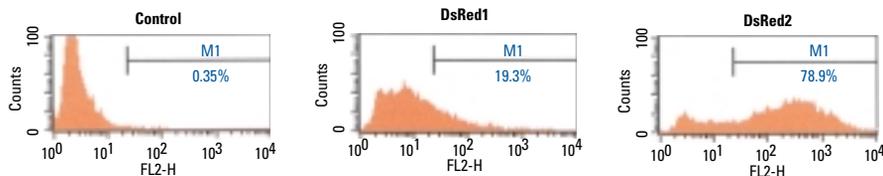
The mutations have also reduced the time from transfection of your cells to detection of fluorescence. DsRed2 only needs 24 hours for the production of sufficient fluorescence to afford accurate and consistent results. Our

**Table 1: Comparison of DsRed1 and DsRed2**

	DsRed2	DsRed1
Excitation maximum	558 nm	558 nm
Emission maximum	583 nm	583 nm
Signal-to-noise ratio	high	high
Time to detection	24 hr	48 hr
Aggregation	very low	high
Toxicity	low	moderate
Oligomerization status	tetramer	tetramer

original DsRed1 required more than 48 hours to detect sufficient fluorescence. In addition, the relative fluorescence intensity of DsRed2 is greater than DsRed1 (Figure 3A). Figure 3B illustrates that transfection efficiency is unaffected by the mutations made to our red fluorescent protein. In the 24 hours following transfection of 293 cells, a much higher number of cells expressing DsRed2 were visible. By 72

# Living Colors™ DsRed2...continued



**Figure 2. Reduced toxicity of DsRed2 to mammalian cell lines.** Following transfection of 293 cells with either pDsRed2-N1 or pDsRed1-N1, we cultured cells for two weeks in the presence of G418. We then sorted both populations via FACS to remove any nonfluorescent cells and separately maintained each population emitting fluorescence for an additional four weeks in the presence of G418. FACS analysis revealed that the original population of DsRed2-expressing cells maintained a high percentage of positive cells and fluorescence intensity during the four weeks of culture, whereas the DsRed1 cell population showed a decrease in both average intensity and number of positive cells. M1: region chosen for initial sorting of fluorescent cells. Numbers in blue indicate the percentages of the total cell populations in the M1 regions.

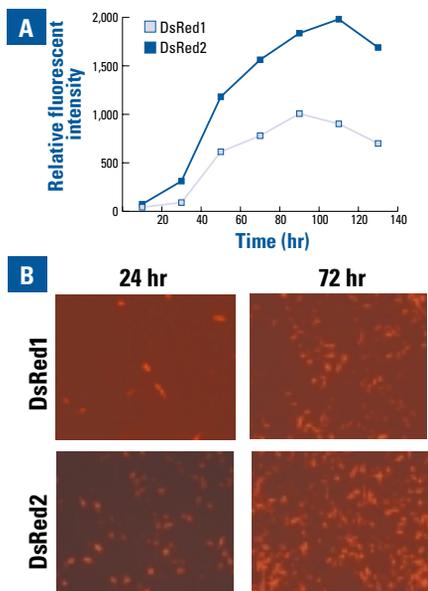
hours after transfection, both cell populations have similar numbers of red fluorescent cells.

### Same spectral properties as DsRed1

The mutations made to create DsRed2 do not alter excitation and emission spectra, so you can detect DsRed2 like DsRed1. DsRed2 has a high signal-to-noise ratio with an emission maximum of 583 nm. Since the emission spectrum of our red fluorescent protein is shifted from the EGFP variants, separating cells expressing DsRed2 from cells expressing any of our EGFP variants is easily accomplished using flow cytometry or fluorescence microscopy.

The modifications made to DsRed2 do not affect the region of the protein used to generate the Living Colors™ D.s. Peptide Antibody (#8370-1, 2). Thus, you can still use this antibody for your immunoprecipitations, Western blotting, or immunohistochemical assays.

We offer four DsRed2 vectors: a source vector (pDsRed2), a promoterless vector (pDsRed2-1), an N1 fusion vector (pDsRed2-N1) for N-terminal tagging, and a C1 fusion vector (pDsRed2-C1) for C-terminal tagging. Each fusion protein should be empirically tested to determine if steric interference affects its function. Such experiments should identify the most appropriate location for the fusion of DsRed2 to the target protein.



**Figure 3. Fluorescence develops within 24 hours of transfection using pDsRed2-N1.** Panel A. Comparison of the relative fluorescence intensity detected in 293 cells expressing DsRed1 or DsRed2. Following transient transfection with pDsRed2-N1 or pDsRed1-N1 (10 µg plasmid DNA/ 2 x 10<sup>6</sup> cells), we measured fluorescence periodically by FACS analysis (FACS Caliber, BD Biosciences Immunocytometry Systems). The average red fluorescence measured in cells expressing DsRed2 was approximately two-fold higher than cells expressing DsRed1. Panel B. Comparison of the relative quantities of HeLa cells expressing DsRed1 or DsRed2 at 24 and 72 hr after transfection. We plated HeLa cells on coverslips, cultured them overnight, and transfected them with 0.75 µg of pDsRed2-N1 or pDsRed1-N1 per coverslip using a lipid-based transfection agent. After 24 hr and 72 hr, we fixed the cells and photographed images under a 10X objective. A much higher number of cells expressing DsRed2 were visible 24 hr after transfection. By 72 hr after transfection, the numbers of red fluorescent cells were similar.

Product	Size	Cat. #
pDsRed2 Vector	20 µg	6943-1
pDsRed2-1 Vector	20 µg	6944-1
pDsRed2-N1 Vector	20 µg	6973-1
pDsRed2-C1 Vector	20 µg	6974-1

NEW!

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