

Living Colors® Red Fluorescent Protein

The only red fluorescent protein for expression studies

- Exclusively available from CLONTECH
- Ideal for *in vivo*, multiple color labeling
- Virtually eliminates background fluorescence
- Highly specific antibody available

CLONTECH introduces **Living Colors® Red Fluorescent Protein**¹—the only commercially available red fluorescent protein (RFP) for expression studies. This unique protein (DsRed) was isolated from the IndoPacific sea anemone relative *Discosoma striata* (1). It has a vivid red fluorescence (Figure 1), making it ideal for multiple labeling with CLONTECH's Living Colors enhanced green fluorescent protein (EGFP) variants. DsRed's excitation and emission peaks produce a high signal-to-noise ratio with excellent *in vivo* transmission.

DsRed represents a new direction for our Living Colors product line. Until now, GFP isolated from *Aequorea victoria* has been the cornerstone of Living Colors reporters, available in blue, cyan, green, and yellow-green variants. With the addition of DsRed, the Living Colors product line now spans an even broader range of fluorescent proteins for monitoring gene expression *in vitro*, *in vivo*, and in real time. We offer a wild-type DsRed source vector, two human codon-optimized DsRed1 mammalian expression vectors (2, 3), and a highly specific peptide antibody for a comprehensive line of expression and detection tools.

Unique, red spectra for optimal labeling
As Table I shows, DsRed fluoresces brightly with a maximum emission at 583 nm (1), red-shifted by more than 50 nm from the other Living Colors fluorescent proteins. This wavelength is easily detected using common rhodamine or propidium iodide filters. DsRed is efficiently excited by a 488-nm argon laser, making it well suited for use in flow cytometry or scanning confocal microscopy.

DsRed's spectrum virtually eliminates the autofluorescence that interferes with visualization

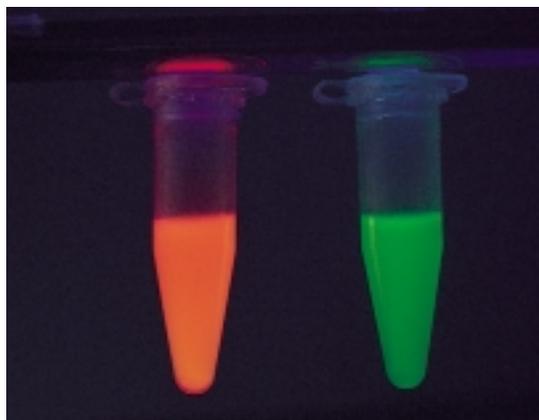


Figure 1. Bright fluorescence of DsRed and GFP.

of GFP variants. In some cases, autofluorescence from the media, culture dish, or cellular components is indistinguishable from a GFP variant's fluorescence; this noisy background can make it difficult to visualize low-abundance proteins or dimly emitting fusions. Because DsRed fluoresces outside the range of most autofluorescence, it stands out sharply from the background, giving the best possible signal-to-noise ratio.

For *in vivo* labeling, DsRed offers a further advantage over other Living Colors proteins. Tissues specifically absorb the short-wavelength blues and greens of GFP variants, reducing the amount of light that transmits *in vivo*. Because tissues absorb less energy at longer wavelengths, DsRed's fluorescence transmits clearly through tissues. This characteristic makes DsRed a valuable tool for label-

ing tissues that have been hard to label with GFP variants.

Well suited for multiple labeling

DsRed's emission peak is clearly distinct from all EGFP variants (Figure 3). It can be easily distinguished from the EGFP variants by fluorescence microscopy or flow cytometry using the appropriate filters. This spectrum makes DsRed ideal for colabeling with any Living Colors protein, particularly with EGFP (Figure 7). Together, DsRed and EGFP are excellent tools for a variety of applications, such as labeling two proteins in a single cell or tissue, monitoring gene expression simultaneously from two different reporters, or analyzing mixed cell population by flow cytometry. For triple labeling such as in Figure 2, we recommend DsRed, ECFP, and EYFP. For more information about filter sets, see the insert "How to Detect DsRed" on page 4.

Table I: Characteristics of all Living Colors® Fluorescent Proteins

	Excitation max (nm)	Emission max (nm)	Extinction coefficient	Quantum yield
DsRed	558	583	22,500	0.29 ^a
EYFP	514	527	84,000	0.61
EGFP	489	508	55,000	0.60
ECFP	434	477	26,000	0.40
EBFP ^b	380	440	31,000	0.18

^a Relative to EGFP

^b Rapid photobleaching

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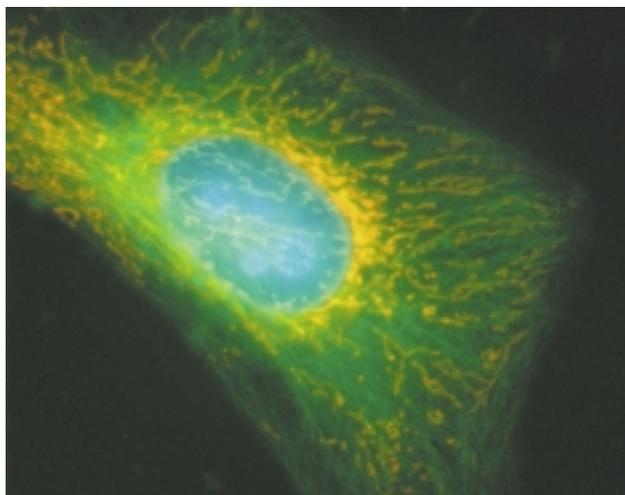


Figure 2. Triple labeling with DsRed1, ECFP, and EYFP. HeLa cells were transiently transfected with pECFP-Nuc (#6904-1), pEYFP-Tub (#6118-1), and pDsRed1-Mito (n/a), which label the nucleus, tubulin, and mitochondria, respectively. The cells were incubated at 37°C for 48 hr, fixed in 3.7% formaldehyde in PBS, and observed by fluorescence microscopy using a Zeiss Axioskop. The images were taken with Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc, a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific), and MetaMorph Software (Universal Imaging Corp.). Individual images were overlaid and pseudocolored. The overlap of EYFP and DsRed gives a bright yellow color.

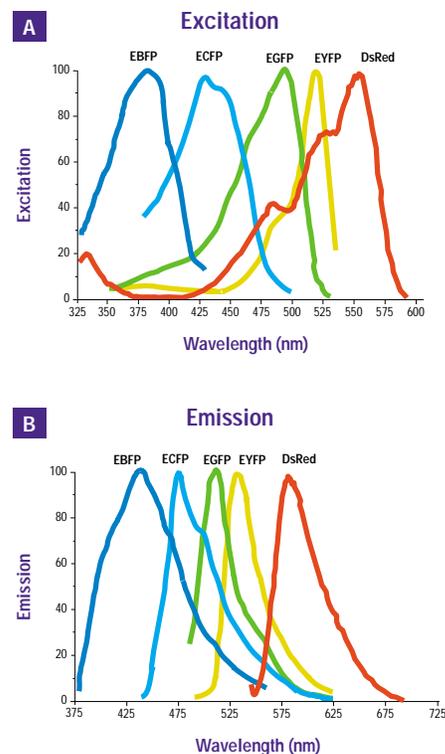


Figure 3. Excitation and emission spectra for all Living Colors® fluorescent proteins.

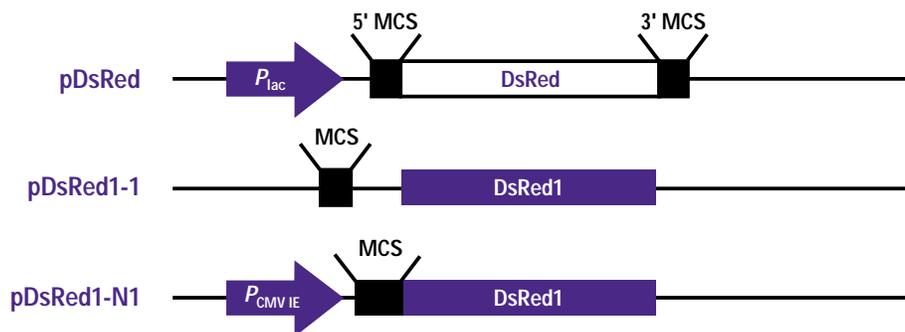


Figure 4. Vector maps for pDsRed, pDsRed1-1, and pDsRed1-N1. pDsRed contains the wild-type DsRed coding sequence flanked by MCSs for cloning into an expression vector. The vector is Amp resistant for selection in bacterial cells. pDsRed1-1 is a promoterless version of the human codon-optimized variant. It contains an MCS upstream from the coding region for inserting promoter or enhancer elements. pDsRed1-N1 is a human codon-optimized variant that uses the strong CMV IE promoter. This vector expresses DsRed in mammalian cells, or allows you to create an N-terminal fusion. Both pDsRed1-1 and pDsRed1-N1 are Kan and Neo resistant for selection in bacterial or eukaryotic cells.

DsRed—the newest member of the Living Colors® family
As with all Living Colors proteins, DsRed requires no additional cofactors or substrates for fluorescence—you simply excite fluores-

cence with the appropriate wavelength of light. Since expressing DsRed does not require cell lysis, you can monitor events as they naturally occur. As a result, you can use DsRed as a fluorescent tag to follow protein localization (4), as

a transcription marker for cell lineage studies, or in transgenic studies.

Versatile vectors for expression studies
We offer three vectors for DsRed expression, shown in Figure 4:

- **pDsRed1-N1** constitutively expresses the human codon-optimized variant from the strong CMV promoter. Unmodified, this vector expresses high levels of DsRed1 for mammalian studies. It also allows you to create N-terminal fusions by cloning proteins of interest into the MCS.
- **pDsRed1-1** is a promoterless vector containing the human codon-optimized variant. With this vector, you can study promoter or enhancer elements inserted into the MCS upstream of the DsRed1 coding region. This vec-

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tor does not express DsRed1 unless a promoter is inserted into the MCS.

- **pDsRed** contains the wild-type coding sequence of DsRed for expression in nonmammalian applications. You can easily excise the coding sequence using the flanking MCSs or amplify it by PCR and insert it into your vector of choice.

In the human codon-optimized variant, we made silent base substitutions to the wild-type sequence, replacing codons that are not translated efficiently in mammalian systems with more preferred codons (2, 3). With the addition of a Kozak consensus translation initiation site (5), this sequence is optimized for enhanced translation efficiency in mammalian

cells. For nonmammalian applications, we recommend the pDsRed Vector which contains the unmodified wild-type sequence. See inset on page 4 for information on Living Colors nonmammalian expression vectors.

Highly specific antibody

We also offer the highly specific **Living Colors D.s. Peptide Antibody** for Western blotting, immunoprecipitations, or histochemical assays. This sensitive affinity-purified peptide antibody can detect the wild-type DsRed and human codon-optimized DsRed1 variants. Because DsRed is derived from a different organism, the antibody does not cross react with any *A. victoria* GFP variants.

To show the antibody's specificity, we transfect cells with pDsRed1-N1, then used Living

Colors D.s. Peptide Antibody in a Western analysis of the whole cell lysate (Figure 9). The antibody detected DsRed from as little as 0.2 µg of cell lysate with very low background.

Complete information provided

All DsRed vectors are supplied with complete sequence information and the Living Colors User Manual Volume II (PT3404-1), which includes protocols for the expression and detection of DsRed. Vector maps are available at vectors.clontech.com.

See page 5 for ordering information.

How to Detect DsRed

Fluorescence microscopy

Although there are currently no optimized filter sets for detecting DsRed, we have seen excellent results using standard filters for either rhodamine or propidium iodide to detect the red fluorescence (Figure 5). For dual labeling with DsRed and EGFP such as in Figure 7, we recommend a rhodamine or propidium iodide filter to detect DsRed and a FITC filter or any EGFP-optimized filter. You can triple-label cells, as in Figure 2, with DsRed, ECFP, and EYFP.

DsRed emits to a low extent in response to wavelengths used to excite cyan (ECFP), green (EGFP), and green-yellow (EYFP) emission channels, which could lead to some bleedthrough. This bleedthrough is small and should not affect multiple labeling such as that shown in Figures 2 and 7. If the bleedthrough does become apparent, you can compensate with shorter exposure times, background subtraction, or appropriate threshold settings when using digital imaging.



Figure 5. Cells expressing DsRed. HEK 293 cells were transiently transfected with pDsRed1-N1, fixed, and observed as in Figure 2. The image was taken using Omega filter set XF35 (Exciter, 535DF35; Dichoic, 560DCLIP; Emitter 635DF55), commonly used for detecting propidium iodide.

Flow cytometry

DsRed is efficiently excited by the 488 nm argon laser used in many standard flow cytometry machines. You can detect the signal in the conventional FL2 channel, as Figure 6 shows.

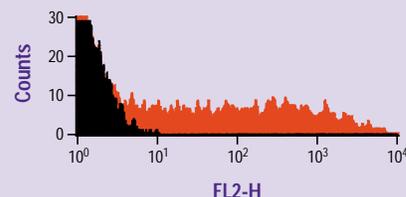


Figure 6. Detection of DsRed1-expressing cells by flow cytometry. HEK 293 cells were transiently transfected with pDsRed1-N1. Three days after transfection, cells were collected and analyzed by FACS using 488-nm excitation. Emission was detected in the FL2 585/42 detection channel. DsRed is shown in red. The control nontransfected cells are shown in black.

New filter sets optimized for detecting DsRed are currently being developed by Omega Optical, Inc. and Chroma Technology Corp. Please contact Omega (802-254-2690) and Chroma (802-257-1800) for more information.

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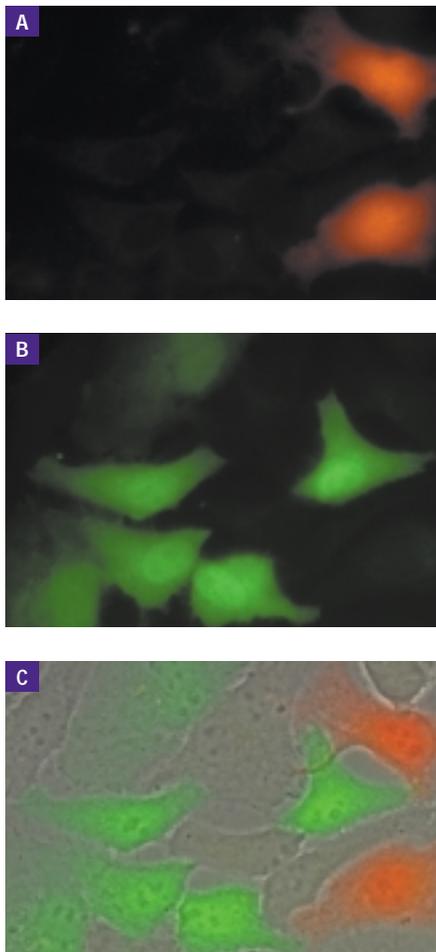


Figure 7. A mixed population of cells expressing DsRed or EGFP. HeLa cells were transiently transfected with either pDsRed1-N1 or pEGFP-C1 (#6084-1), fixed, and observed as described in Figure 2. The images were taken with Chroma filter set 31002 (TRITC) for DsRed or Chroma filter set 31001 (FITC) for EGFP. **Panel A.** Cells expressing DsRed1. **Panel B.** Cells expressing EGFP. **Panel C.** The images from Panels A and B were overlaid on a phase contrast image of the same field.

References

1. Mikhail, V., et al. (1999) *Nature Biotechnol.*, in press.
2. Living Colors Enhanced GFP Vectors (April 1996) *CLONTECHniques XI(2):2-3*.
3. Haas, J., et al. (1996) *Curr. Biol.* 6:315-324.
4. Rizzuto, R., et al. (1996) *Curr. Biol.* 6:183-188.
5. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125-8148.

Living Colors® Vectors for Nonmammalian Expression Studies

CLONTECH offers fluorescent protein variants that are optimized for bright fluorescence and high expression, but are not human codon optimized. These variants are ideal for expression studies in nonmammalian systems such as bacteria, yeast, plants, *Xenopus*, and *C. elegans*. Bacterial cells expressing three of these variants are shown in Figure 8.



Figure 8. DsRed, GFPuv, and BFP2 fluorescence. Bacterial cells transfected with pDsRed, pGFPmut3.1, or pBFP2 were streaked individually on LB/amp plates.

- **pGFPuv Vector (#6079-1)** contains mutations that both increase brightness 18-fold over wild type when excited in UV light and increase translation efficiency in *E. coli*. It has excitation and emission peaks of 395 nm and 509 nm.
- **pGFPmut3.1 Vector (#6039-1)** is optimized for bright fluorescence, efficient protein folding, and chromophore function. It has a red-shifted excitation peak of 501 nm and an emission peak of 511 nm.
- **pGFP (ASV, AAV, or LVA) Vector (#K6002-1)** is a set of destabilized variants derived from GFPmut3.1. Each contains a C-terminal tag that targets the protein for degradation. Half lives vary from host to host. In *E. coli*,

GFP (ASV)=110 min, GFP (AAV)=60 min, and GFP (LVA)=40 min. Each has the same excitation and emission peaks as GFPmut3.1.

- **pBFP2 Vector (#6038-1)** is a blue fluorescent variant of GFP with mutations to enhance the brightness and solubility of the protein. It has excitation and emission peaks of 380 nm and 440 nm.
- **pDsRed Vector (#6923-1)** is the wild-type DsRed protein. It has excitation and emission peaks of 558 nm and 583 nm.

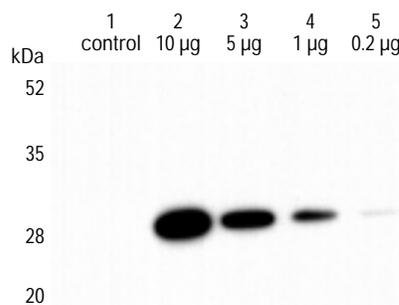


Figure 9. Living Colors® D.s. Peptide Antibody provides a strong, specific signal and high sensitivity. HEK 293 cells were transiently transfected with pDsRed1-N1. Whole cell lysate was then subjected to Western analysis using affinity-purified Living Colors D.s. Peptide Antibody. Lane 1 contains 10 µg of nontransfected cell lysate. The remaining lanes contain the indicated amount of transfected cell lysate.

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How to Obtain DsRed Vectors

Not-For-Profit Entities:

Orders may be placed in the normal manner by contacting your local representative or CLONTECH Customer Service at either 800-662-2566 (CLON) or 650-424-8222, extension 1. No license agreement is necessary.

For-Profit Entities, Non-commercial Use:

Before placing an order for one of our new DsRed vectors, a copy of the Royalty-Free Research and Site License agreement must be obtained from either our web site or by contacting your local representative. This document outlines the conditions under which DsRed vectors can be used for non-commercial purposes. After you have reviewed the conditions of the agreement and signed and returned the document to CLONTECH, you will be able to place an order for any DsRed vector. No license fee will be required.

For any Commercial Use:

Please contact the Product Manager for Cell Biology at extension 7816 (at either 800-662-2566 or 650-424-8222).

Product	Size	Cat. #
pDsRed1-N1 Vector	20 µg	6921-1
pDsRed1-1 Vector	20 µg	6922-1
pDsRed Vector	20 µg	6923-1
Living Colors D.s. Peptide Antibody	20 µg 100 µg	8370-1 8370-2

NEW!

Notice to Purchaser

[†] Patent pending

Use of Living Colors DsRed is limited to research purposes only and may not be sold, modified for resale, or used for commercial purposes. Commercial use of DsRed requires an alternative license, the terms of which can be obtained upon inquiry to the Product Manager for Cell Biology at 800-662-2566 or 650-424-8222 extension 7816. CLONTECH is in the process of patenting various aspects of the DsRed technology.