

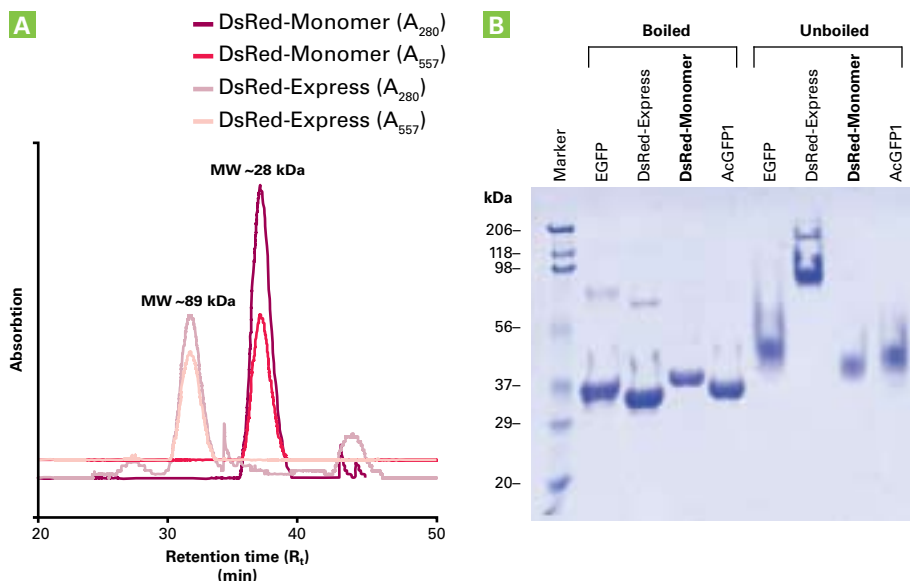
# Living Colors® DsRed-Monomer Fluorescent Protein

## An ideal red fusion tag to study proteins in live cells

- Ideal for fusion tag applications
- Validated with a wide variety of fusion proteins
- Perfect for dual labeling with AcGFP1 green monomer

The **DsRed-Monomer Fluorescent Protein** is a newly engineered mutant of our red fluorescent protein from *Discosoma* sp. reef coral. DsRed-Monomer is ideal for multicolor applications in flow cytometry and fluorescence microscopy. Earlier versions of the DsRed protein have been limited in their utility as fusion tags because of their tetrameric structure. We have created a new monomeric version of DsRed, with 45 amino acid substitutions, that retains spectral properties similar to DsRed-Express. The DsRed-Monomer protein is extremely stable, allowing you to monitor fluorescence over extended periods of time. The chromophore matures rapidly and is readily detected in 12 hours.

The use of DsRed-Monomer as a fusion tag has been validated with a wide variety of proteins with diverse functions and subcellular locations. DsRed-Monomer is well tolerated by mammalian cells, and has been successfully used to create stably transfected, clonal cell lines. Like all Living Colors Fluorescent Proteins, DsRed-Monomer can be detected in cells without adding cofactors or substrates, making it a valuable, noninvasive tool for investigating biological events in living cells (1–3).



**Figure 1. Living Colors DsRed-Monomer is a monomeric protein.** **Panel A.** Recombinant DsRed-Express and DsRed-Monomer (100  $\mu$ g) were analyzed by FPLC gel filtration chromatography. Overall absorption ( $A_{280}$ ) and chromophore excitation ( $A_{557}$ ) of the eluted material were monitored simultaneously. DsRed-Monomer elutes from the column at a retention time (39 min) corresponding to a molecular weight of 28 kDa. The calculated molecular weight of DsRed-Monomer is 26.8 kDa. DsRed-Express is a tetrameric protein that elutes at an earlier retention time (33 min) corresponding to a molecular weight of 89 kDa. **Panel B.** Pseudonative gel analysis of proteins. The oligomeric structures of proteins are preserved during SDS PAGE analysis if samples are kept at 4°C and not boiled prior to loading on a gel. Boiled and unboiled recombinant proteins (7.5  $\mu$ g) were separated by SDS PAGE electrophoresis (12% acrylamide). In both the boiled (denatured) and unboiled (undenatured) samples, DsRed-Monomer and EGFP run as uniform bands of ~30 kDa due to their monomeric structures. The unboiled (undenatured) DsRed-Express runs at a much higher molecular weight than its boiled (denatured) counterpart due to its tetrameric structure.

### A true monomer

The monomeric nature of the DsRed-Monomer protein has been confirmed by several independent methods. When recombinant DsRed-Monomer is analyzed by FPLC gel filtration chromatography, it produces a single uniform peak at a retention time consistent with a 28 kDa molecular weight (Figure 1, Panel A). The elution profile does not display higher molecular weight species and provides strong evidence that DsRed-Monomer is a true monomer. In contrast, recombinant DsRed-Express protein,

because of its tetrameric structure, elutes earlier than DsRed-Monomer. Furthermore, analysis of DsRed-Monomer by pseudonative gel electrophoresis yields a profile that is consistent with a monomeric protein and similar to enhanced green fluorescent (EGFP) and AcGFP1 proteins (Figure 1, Panel B). All of these results agree with the calculated 26.8 kDa molecular weight of DsRed-Monomer based on amino acid sequence.

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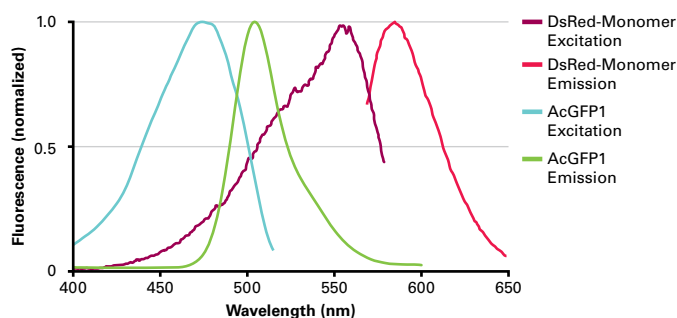
**Table I: Spectral properties of DsRed proteins**

Protein	Excitation Max (nm)	Emission Max (nm)	Time to Detection (hr)	Relative Fluorescent Intensity	Quarternary Structure	Utility as a Reporter	Utility in Fusions
DsRed-Monomer	556	586	12	Bright	Monomer	Fair	Excellent
DsRed-Express	557	579	8–12	Extremely Bright	Tetramer	Excellent	Fair
DsRed2	563	582	24	Extremely Bright	Tetramer	Good	Fair

## Spectral properties

The DsRed-Monomer preserves key spectral features of previous DsRed variants, in particular DsRed-Express. DsRed-Monomer has an excitation maximum of 556 nm and an emission maximum of 586 nm (Table I & Figure 2). Its spectral profile is virtually identical to our other DsRed fluorescent protein variants, which allows DsRed-Monomer to be detected using standard existing filter sets or custom-tailored, optimized sets such as those available from Chroma Technology Corporation (Table II).

Although DsRed-Monomer is somewhat less bright than DsRed-Express (Table 1), it is nevertheless an excellent choice for flow cytometry and fluorescence microscopy imaging (Figures 3–5). As with our other red fluorescent proteins, DsRed-Monomer performs well when multiplexed with compatible fluorescent proteins.



**Figure 2. Fluorescence excitation and emission spectra of DsRed-Monomer and AcGFP1.**

**Table II: Filter sets for DsRed-Monomer detection**

Description	Features
DsRed-Monomer/DsRed2/DsRed-Express Chroma No. 41002c	Exciter HQ545/30x Dichroic Q570LP Emitter HQ620/60m
DsRed-Monomer/DsRed2/DsRed-Express Chroma No. 42005	Exciter HQ540/40x Dichroic Q570LP Emitter HQ600/50m

## Excellent as a fusion tag

Ideally, a fluorescent tag should not interfere with the biological function of the target protein. If the fluorescent protein has a strong tendency to form oligomers, it is more likely to alter or hinder the original function of the tagged protein. Because DsRed-Monomer is a true monomer, it is the optimal choice for use as a red fluorescent fusion tag. When expressed in mammalian cells, the protein is highly soluble and homogeneously distributed within the cytosol, with no detectable aggregation (Figure 4).

Use of DsRed-Monomer as a fusion tag was validated by expressing it as fusions with a large panel of diverse proteins and monitoring the localization of the resulting tagged proteins (Figure 5). All the tested proteins localized properly, including actin and Golgi fusions that have not performed well with other, tetrameric, DsRed proteins. The DsRed-Monomer-Actin fusion protein correctly incorporates into the actin filament system—the cytoskeletal network, ruffling edges, and filipodia. These data clearly illustrate the suitability of DsRed-Monomer as a fusion tag.

Our full range of DsRed-Monomer vectors, including Retroviral Cloning Vectors and prelinearized In-Fusion™ Ready Vectors, provides you with powerful tools to address your specific application needs.

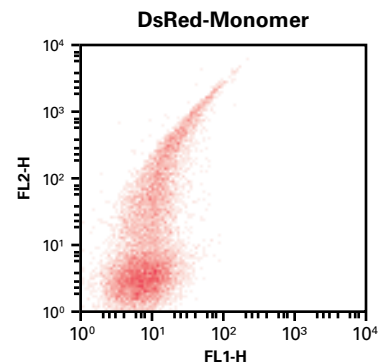
You can monitor DsRed-Monomer easily with our Living Colors DsRed Polyclonal antibody (4; Cat. No. 632496). It is a rabbit polyclonal serum, generated against full-length DsRed-Express protein, that recognizes wild-type DsRed and all its variants. It detects DsRed-Monomer in both Western blot and immunoprecipitation applications.

## Want more details?

Visit [www.clontech.com](http://www.clontech.com) for more information about our Living Colors Fluorescent Proteins. Information on Chroma Technology Corporation filter sets can be found on our web site and at [www.chroma.com](http://www.chroma.com).

## References

1. Reef Coral Fluorescent Protein Vectors (July 2003) *Clontechniques XVIII*(3):6–7.
2. Matz, M. V., et al. (1999) *Nature Biotechnol.* 17:969–973. *Erratum in: Nature Biotechnol.* (1999) 17:1227.
3. BD Living Colors™ DsRed-Express (July 2003) *Clontechniques XVII*(3):16.
4. BD Living Colors™ DsRed Polyclonal Antibody (Jan 2003) *Clontechniques XVIII*(1):11.

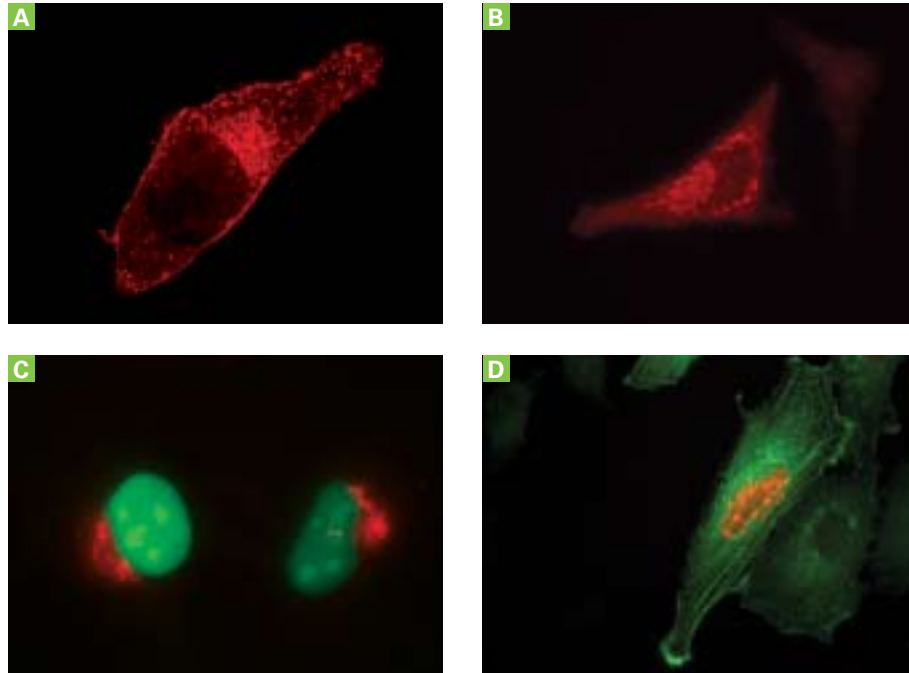


**Figure 3. Cells expressing DsRed-Monomer are easily detected by standard BD FACS™ analysis.** HEK 293 cells were transfected with pDsRed-Monomer-N1 and analyzed using the 568 nm laser line of the BD FACSVantage™ SE Flow Cytometry System 24 hr post-transfection.



**Figure 4. DsRed-Monomer is soluble when expressed in mammalian cells.** HeLa cells were transfected with pDsRed-Monomer-N1 and fixed in 4% paraformaldehyde 24 hr post-transfection. DsRed-Monomer displays an even, consistent, and homogeneous distribution.

# Living Colors® DsRed-Monomer...continued



**Figure 5. DsRed-Monomer performs well in protein fusions and multicolor applications.** Various DsRed-Monomer fusions were transiently transfected in HeLa cells and visualized by fluorescence microscopy. Cells were fixed in 4% paraformaldehyde 24 hr post-transfection. **Panel A.** DsRed-Monomer-Caveolin fusion. **Panel B.** DsRed-Monomer-Rab5 GDP/GTP GTPase exchange factor homologue fusion. **Panel C.** DsRed-Monomer-Golgi (trans Golgi stack) and AcGFP1-Nuc (nucleus). **Panel D.** DsRed-Monomer-Lamin B fusion and AcGFP1-Actin.

#### Related Products

- Basic Fluorescent Protein Vectors
- Promoterless Fluorescent Protein Vectors
- N-Terminal Fluorescent Protein Vectors
- C-Terminal Fluorescent Protein Vectors
- Subcellular Localization Vectors
- IRES Bicistronic Expression Vectors
- Proteasome Sensor Vector
- Fluorescent Timer Vectors
- Destabilized Fluorescent Protein Vectors
- Recombinant Fluorescent Proteins
- Living Colors Antibodies
- Living Colors Sequencing Primers
- Living Colors Cell Lines
- Fluorescent Proteins: DsRed-Monomer, DsRed2, DsRed-Express, AsRed2, HcRed1, AmCyan1, ZsYellow1, ZsGreen1 & AcGFP1

Several additional vectors are available. Please see our website at [www.clontech.com](http://www.clontech.com) for details.

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Living Colors® DsRed-Monomer Products	Size	Cat. No.	Price
pDsRed-Monomer Vector	20 µg	632467	*\$516.00
pDsRed-Monomer-N1 Vector	20 µg	632465	*\$516.00
pDsRed-Monomer-C1 Vector	20 µg	632466	*\$516.00
pDsRed-Monomer-Actin Vector	20 µg	632479	*\$516.00
pDsRed-Monomer-Golgi Vector	20 µg	632480	*\$516.00
pDsRed-Monomer-F Vector	20 µg	632493	*\$516.00
pDsRed-Monomer-Hyg-N1 Vector	20 µg	632494	*\$516.00
pDsRed-Monomer-Hyg-C1 Vector	20 µg	632495	*\$516.00
pDsRed-Monomer-N In-Fusion Ready Vector	1 µg	632498	**\$356.00
pDsRed-Monomer-C In-Fusion Ready Vector	1 µg	632499	**\$356.00
pRetroQ-DsRed Monomer-N1 Vector	20 µg	632507	*\$516.00
pRetroQ-DsRed Monomer-C1 Vector	20 µg	632508	*\$516.00
pDsRed-Monomer-Mem Vector	20 µg	632512	*\$516.00
pDsRed-Monomer-Mem Hyg Vector	20 µg	632513	*\$516.00
pDsRed-Monomer-F Hyg Vector	20 µg	632514	*\$516.00
rDsRed-Monomer Protein	100 µg	632503	\$292.00

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\* Not-For-Profit entities.  
For-Profit entities: \$1,331.00.

\*\* Not-For-Profit entities.  
For-Profit entities: \$920.00.