BD Living Colors™ DsRed-Monomer Fluorescent Protein

An ideal red fusion tag to study proteins in live cells

- **First commercially available monomeric red fluorescent protein**
- **Ideal for fusion tag applications**
- **Validated with a wide variety of fusion proteins**
- **Perfect for dual labeling with AcGFP1 green monomer**

The BD Living Colors™ 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 (e.g. BD FACS™ analysis) 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 a total of 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 BD Living Colors™ Novel Fluorescent Proteins (NFPs), 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).

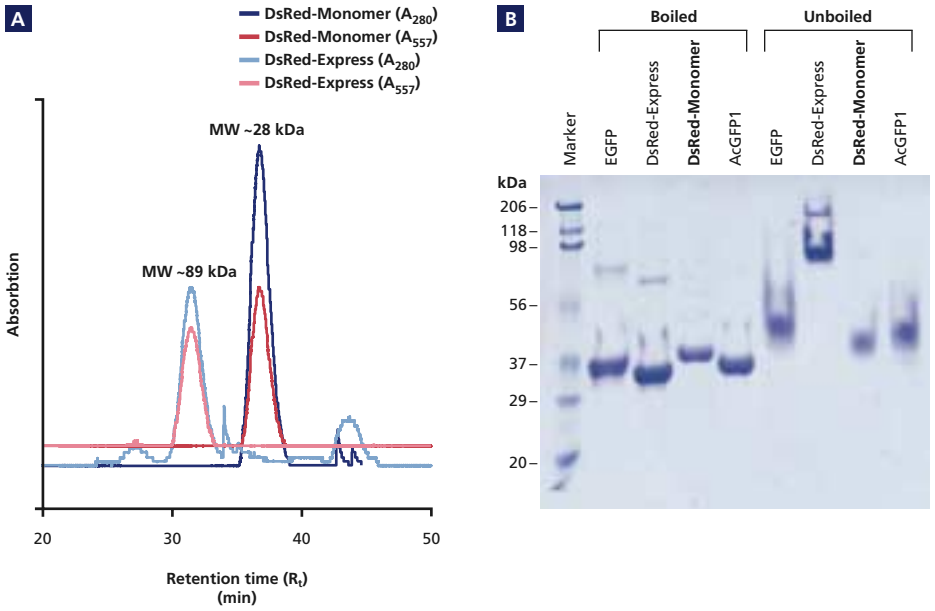

Figure 1. BD Living Colors™ DsRed-Monomer is a monomeric protein. Panel A. Recombinant DsRed-Express and DsRed-Monomer (100 µg) were analyzed by FPLC gel filtration chromatography. Overall absorption (A_{280}) and chromophore excitation (A_{557}) of the eluted material was monitored simultaneously. DsRed-Monomer 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 structure 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 µg) 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 tetrametric 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 DsRed-Express 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-Monomer protein

Protein	Excitation Max (nm)	Emission Max (nm)	Time to detection (hr)	Relative fluorescent intensity	Quaternary structure	Utility as a reporter	Utility in fusions
DsRed-Monomer	556	586	12	Bright	Monomer	+	+++
DsRed2	563	582	24	Extremely bright	Tetramer	+++	+
DsRed-Express	557	579	8–12	Extremely bright	Tetramer	+++	+

Note: +++ = Excellent performance. ++ = Good performance. + = Fair performance.

Spectral properties

One of the major goals accomplished in creating the DsRed-Monomer was the preservation of 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 virtually identical spectral profile to our other DsRed fluorescent protein variants allows DsRed-Monomer to be detected using standard existing filter sets. This also includes 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 & Figure 3*), it is nevertheless an excellent choice for fluorescence microscopy imaging and flow cytometry (*Figures 4–6*). As with our other red fluorescent proteins, DsRed-Monomer performs well when multiplexed with compatible fluorescent proteins.

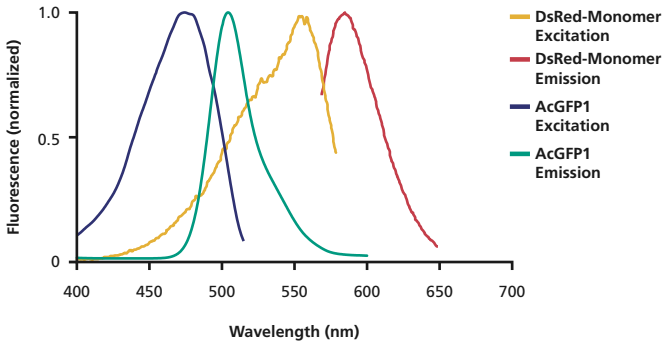

Figure 2. Fluorescence excitation and emission spectra of DsRed-Monomer and AcGFP1.

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

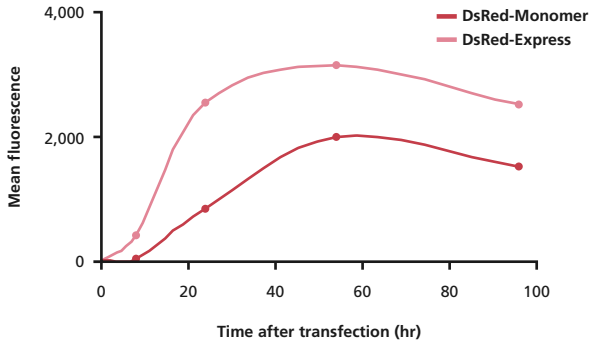

Figure 3. Time course of DsRed-Monomer fluorescence intensity. HEK 293 cells were transfected with the construct indicated and analyzed using the 568 nm laser line of the BD FACSVantage™ SE Flow Cytometry System at the indicated time points. The fluorescence intensity of DsRed-Monomer protein is somewhat lower in comparison to DsRed-Express protein when analyzed by flow cytometry, but appears very bright in fluorescence microscopy. Note that both proteins achieve maximal fluorescence intensity at approximately the same time point post-transfection.

BD Living Colors™ DsRed-Monomer...continued

Excellent as a fusion tag

Ideally, when using a fluorescent tag to label a protein of interest, the tag itself 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 5*).

Use of DsRed-Monomer as a fusion tag was validated by expressing it as a fusion with a large panel of diverse proteins and monitoring the localization of the resulting tagged protein (*Figure 6*). All the proteins tested localized properly, including actin and Golgi fusions that have not performed well with earlier 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.

The actin and Golgi functional vectors allow you to overcome the challenges previously associated with studying these components in the past. These subcellular localization vectors, along with BD Creator™ Acceptor vectors featuring DsRed-Monomer, will soon be available. The Creator vectors allow both N- and C-terminal cloning of DsRed-Monomer into our versatile BD Creator™ System (4). This full range of DsRed-Monomer vectors provides you with powerful tools to address your specific application needs.

Monitoring DsRed-Monomer is easily accomplished by using our BD Living Colors™ DsRed Polyclonal and Monoclonal antibodies (5; **Cat. Nos. 632397, 632392 & 632393**). They detect DsRed-Monomer in both Western blot and immunoprecipitation applications (*Table III*).

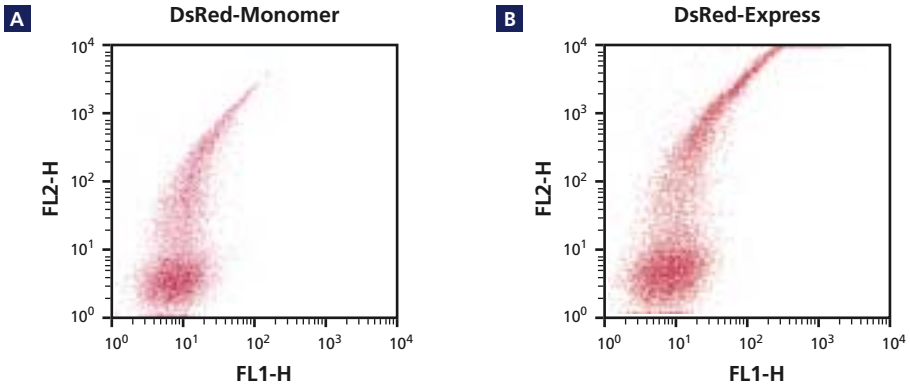

Figure 4. Cells expressing DsRed-Monomer are easily detected by standard BD FACS™ analysis. HEK 293 cells were transfected with the construct indicated and analyzed using the 568 nm laser line of the BD FACSVantage™ SE Flow Cytometry System 24 hr post-transfection. **Panel A.** pDsRed-Monomer-N1. **Panel B.** pDsRed-Express-N1.

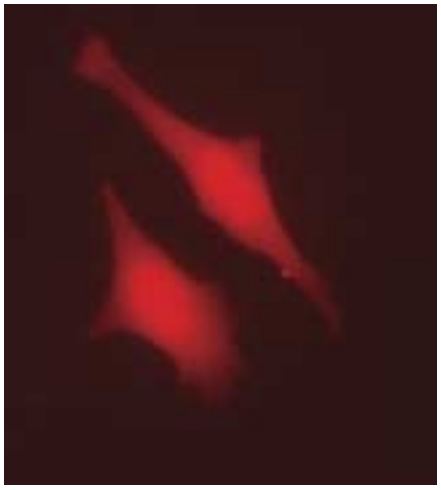

Figure 5. DsRed-Monomer is soluble when expressed in mammalian cells. HeLa cells were transfected with pDsRed-Monomer-N1 and fixed in 4% paraformaldehyde 24 hr post-transfection. DsRed-Monomer displays an even, consistent, and homogeneous distribution.

Table III: BD Living Colors™ Antibodies for DsRed-Monomer

Antibody name	Cat. No.	Antibody characteristics	Application	
			Western	IP
DsRed Polyclonal Antibody	632397	Rabbit polyclonal serum generated against DsRed-Express protein	+++	+++
DsRed Monoclonal Antibody	632392 632393	Affinity-purified mouse monoclonal	+	+

Note: IP = Immunoprecipitation.
+++ = Excellent performance. + = Fair performance.

BD Living Colors™ DsRed-Monomer...continued

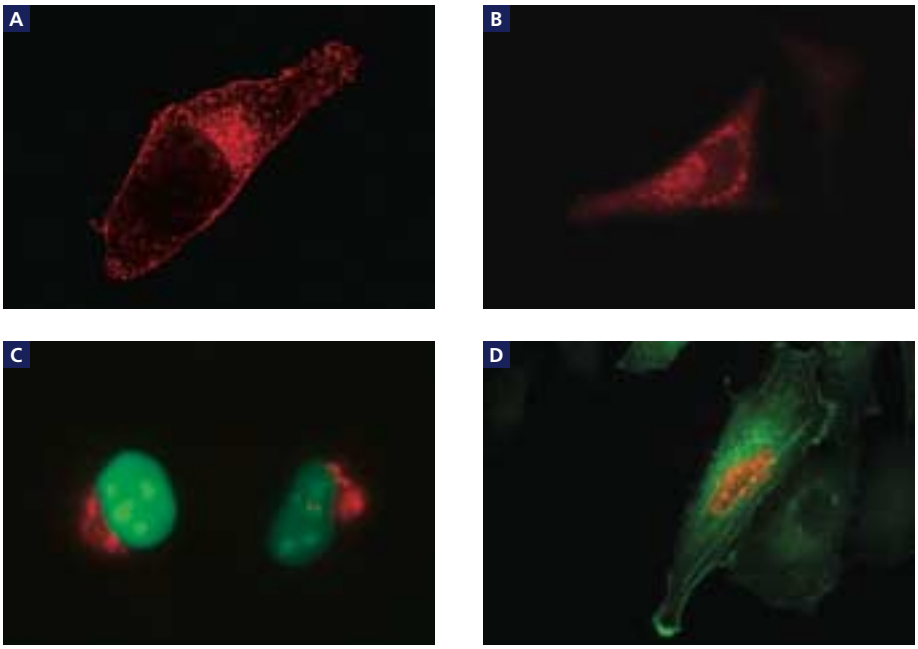

Figure 6. DsRed-Monomer performs well in protein fusions and multicolor applications. Various DsRed-Monomer fusions (constructed with BD Creator™ technology) were transiently transfected in HeLa cells and visualized by fluorescence microscopy. Cells were fixed in 4% paraformaldehyde 24 hr post-transfection. **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.

BD Clontech Fluorescent Proteins are the only commercially available green and red monomeric proteins suitable for dual color fusion applications.

Want more details?

Visit **www.bdbiosciences.com/clontech** for more information about our BD Living Colors™ Fluorescent Proteins. Information on Chroma Technology Corporation filter sets can be found on our web site and at **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. Creator™ System Overview (October 2001) *Clontechniques* **XVI**(4):5–6.
5. BD Living Colors™ DsRed Polyclonal Antibody (Jan 2003) *Clontechniques* **XVIII**(1):11.

Coming Soon!

DsRed-Monomer actin and Golgi subcellular localization vectors, as well as BD Creator™ Acceptor Vectors for N- and C-terminal cloning into our versatile BD Creator System.

Product List

BD Living Colors™ DsRed-Monomer Products

PRODUCT	SIZE	CAT. NO.
pDsRed-Monomer Vector	20 µg	632467
pDsRed-Monomer-N1 Vector	20 µg	632465
pDsRed-Monomer-C1 Vector	20 µg	632466

Related Products

- DsRed Polyclonal Antibody (Cat. No. 632397)
- DsRed Monoclonal Antibody (Cat. Nos. 632392 & 632393)
- pAcGFP1 Vector (Cat. No. 632468)
- pAcGFP1-N1 Vector (Cat. No. 632469)
- pAcGFP1-C1 Vector (Cat. No. 632470)
- AcGFP Vector Set (Cat. No. 632426)
- pIRES2-AcGFP1 Vector (Cat. No. 632435)
- pAcGFP1-Actin Vector (Cat. No. 632453)
- pAcGFP1-Golgi Vector (Cat. No. 632464)
- pAcGFP1-Mito Vector (Cat. No. 632432)
- pAcGFP1-Nuc Vector (Cat. No. 632431)
- pLP-AcGFP1-C Acceptor Vector (632471)
- pLPS-AcGFP1-N Acceptor Vector (632472)

Notice to purchaser

This product is the subject of pending U.S. patents.

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