



BD Living Colors™ Fluorescent Proteins

Investigate biological events in living cells

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BD Living Colors™ Fluorescent Proteins

A multicolor palette of fluorescent reporters

- **Bright fluorescence, proven photostability, fast detection**
- **Well tolerated by mammalian cells**
- **No cofactors or chemical staining required—perfect for live-cell assays**

With the BD Living Colors line, you have access to the largest selection of fluorescent proteins on the market (Table I). Particularly useful are the **Enhanced GFP Color Variants from *Aequorea Victoria***: ECFP (cyan), EGFP (green), and EYFP (yellow). Improved through mutagenesis, these proteins are some of the most widely used reporters in biological research. They can be used not only as tags to track proteins in living cells, but also as reporters to monitor promoter activity and as labels to visualize specific tissues, whole cells or subcellular organelles. Like our Reef Coral Fluorescent Proteins, the GFP color variants have been optimized for brighter emission and faster chromophore maturation, and their genes have been human codon-optimized to enhance their translation in mammalian cells.

Our family of **Reef Coral Fluorescent Proteins (RCFPs)** includes cyan, green, yellow, red, and far-red fluorescent proteins (Figure 1). Like the enhanced color variants of *Aequorea* GFP, RCFPs can be detected in cells and tissues without having to add cofactors or substrates, and they are extremely stable, allowing you to monitor their fluorescence over extended periods. Though not generally recommended for use as protein tags, RCFPs are ideal for monitoring promoter activity, labeling whole cells, and, in some cases, visualizing subcellular organelles (Figure 2). Some RCFPs, such as ZsGreen1 and AmCyan1, are exceptionally bright and photostable, and most mature rapidly *in vivo*, permitting detection within 8–12 hr of transfection. The RCFP group, in addition to having the only red and far-red fluorescent proteins, also boasts the only true yellow fluorescent protein, ZsYellow1, whose emission is ideally positioned between those of ZsGreen1 and AsRed2; in fact, all three proteins can be separated by flow cytometry using a single laser line (488 nm) and common channels of detection. RCFPs are well tolerated by mammalian cells, and have proven to be useful for creating stably transfected cell lines and transgenic organisms (Figure 3).



Figure 1. BD Living Colors™ Reef Coral Fluorescent Proteins viewed under UV light. From left to right, they are AmCyan1, ZsGreen1, ZsYellow1, DsRed2, AsRed2, and HcRed1.

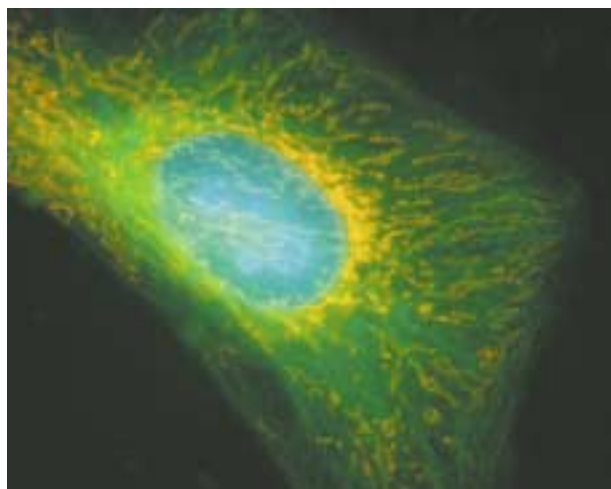


Figure 2. Triple-labeling with BD Living Colors™ proteins. HeLa cells were transiently transfected with pDsRed1-Mito, pEYFP-Tub, and pECFP-Nuc, and fixed in 3.7% formaldehyde in PBS.

EGFP (Enhanced Green Fluorescent Protein) includes chromophore mutations that make it 30–35 times brighter than wild-type GFP (1), permitting detection at levels as low as ~100 nM, equivalent to ~10,000 molecules in the cell cytoplasm (2).

BD Living Colors™ Fluorescent Proteins...continued

Revolutionary reporters for investigating biological events in living cells

- Available in a broad range of colors—cyan, green, yellow, red, and far-red
- Proven performance in a wide variety of prokaryotic and eukaryotic hosts
- Ideal for multiplexing—visualize multiple cellular events simultaneously

BD Living Colors Fluorescent Proteins provide a valuable, non-invasive approach for investigating biological events in living cells and tissues. Ranging in color from cyan to far-red (Table I), these proteins can be used as molecular tags or as independent reporters to visualize, track, and quantify many different cellular processes, including protein synthesis and turnover, protein translocation, gene induction, and cell lineage. Because they require no additional substrates or cofactors for their fluorescence, BD Living Colors Fluorescent Proteins are ideal for use in live cell assays. And because of their distinctive spectra, they can be readily multiplexed—that is, combined for the simultaneous detection of two or more events in the same cell or cell population.



Figure 3. Coexpression of EGFP and DsRed in a zebrafish embryo. The image shows a 30-hour zebrafish embryo coexpressing EGFP driven by the rat GAP-43 promoter and DsRed driven by the *Xenopus* EF 1 α promoter. Photo courtesy of Dr. Y. Kamei and Dr. S. Yuba, Osaka University.

Table I: Comparison of BD Living Colors™ Fluorescent Proteins

Protein	Excitation Max (nm)	Emission Max (nm)	Time to detection (hr)*	Brightness relative to EGFP	Structure	Utility as a reporter	Utility in fusions	Comments
Reef Coral Fluorescent Proteins								
AmCyan1	458	489	8–12	+++	Tetramer	+++	+	Photostable alternative to ECFP
ZsGreen1	493	505	8–12	++++	Tetramer	++++	+	Bright green
ZsYellow1	529	539	8–12	++	Tetramer	+++	+	True yellow emission; ideal for multicolor applications
DsRed-Express	557	579	8–12	+++	Tetramer	+++	++	Preferred DsRed for FACS due to diminished green emission, faster maturation
DsRed2	563	582	24	+++	Tetramer	+++	++	Low aggregation
AsRed2	576	592	8–12	++	Tetramer	+++	+	
HcRed1	588	618	16	+	Dimer	+	+++	Far-red fluorescence; can be multiplexed for four color analysis
<i>Aequorea victoria</i> GFP variants								
ECFP	439	476	8–12	+	Monomer	+	++++	Not as photostable as EGFP, EYFP
EGFP	484	510	8–12	+++	Monomer	+++	++++	
EYFP	512	529	8–12	++	Monomer	+++	++++	Green/yellow

Although RCFPs derive from a different class of organisms, they share structural homology to *Aequorea victoria* green fluorescent protein (GFP). But unlike the color variants of *Aequorea* GFP, RCFPs are unique proteins encoded by distinct genes rather than mutant variants of a single fluorescent protein. With the exception of HcRed1, all RCFPs are believed to have the same tetrameric structure as wild-type DsRed.

* As measured by BD FACST™ analysis using transiently transfected mammalian cell cultures.

Fluorescent Protein Detection & Analysis

Many different options for multispectral imaging

- Monitor two or more cellular events simultaneously
- Visualize protein translocation in relation to other subcellular structures
- Distinctive wavelengths make multicolor separation possible by flow cytometry and fluorescence microscopy

BD Living Colors Proteins offer several different options for single and multicolor analysis (Figure 4). Multicolor analysis, or multiplexing, allows you to simultaneously detect two or more fluorescent proteins in the same cell or cell population. This strategy can be used, for example, to examine interactions between proteins and subcellular organelles, detect the onset of gene expression from distinct promoters, or simply to separate a mixed cell population (Figure 5). The development of optimized filter sets, available from Chroma Technology Corp., and the introduction of our red fluorescent proteins—DsRed2, DsRed-Express, AsRed2, and HcRed1—have greatly extended the benefits of fluorescent protein technology. Researchers can now use standard fluorescence microscopes or flow cytometers (equipped with only a 488-nm laser) to detect as many as three different fluorescent proteins in a mixed cell population. With more advanced set-ups, using more than one laser line, you can separate all four colors (Figure 5; reference 3). Excitation and emission spectra for BD Living Colors Proteins are shown in Figures 6 & 7.

	First Color	Second Color	Third Color
	AmCyan1 ECFP	ZsYellow1	HcRed1
		DsRed2	
		DsRed-Express	
		AsRed2	
	ZsGreen1 EGFP	HcRed1	ZsYellow1
		DsRed2	
		DsRed-Express	
		AsRed2	
	ZsYellow1 EYFP	AmCyan1	HcRed1
		HcRed1	AmCyan1
	DsRed2 DsRed-Express AsRed2	AmCyan1	
		ZsGreen1	
	HcRed1	AmCyan1	ZsYellow1
		ZsYellow1	AmCyan1
		ZsGreen1	

Figure 4. Recommended color combinations for microscopy.

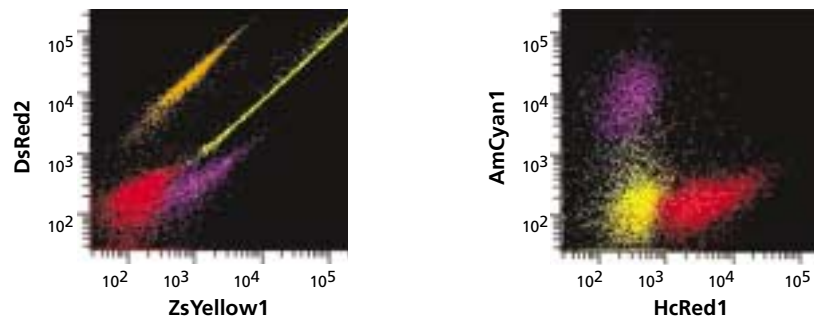


Figure 5. Four-color separation of RCFP-expressing cells using flow cytometry. A mixed population of cells stably expressing either DsRed2, ZsYellow1, HcRed1, or AmCyan1 was separated by flow cytometry with a BD FACSAria™ Cell Sorter using three separate laser lines: 407 nm to excite AmCyan1; 488 nm to excite DsRed2 and ZsYellow1; and 633 nm to excite HcRed1.

Fluorescent Protein Detection & Analysis...continued

Distinct excitation and emission spectra

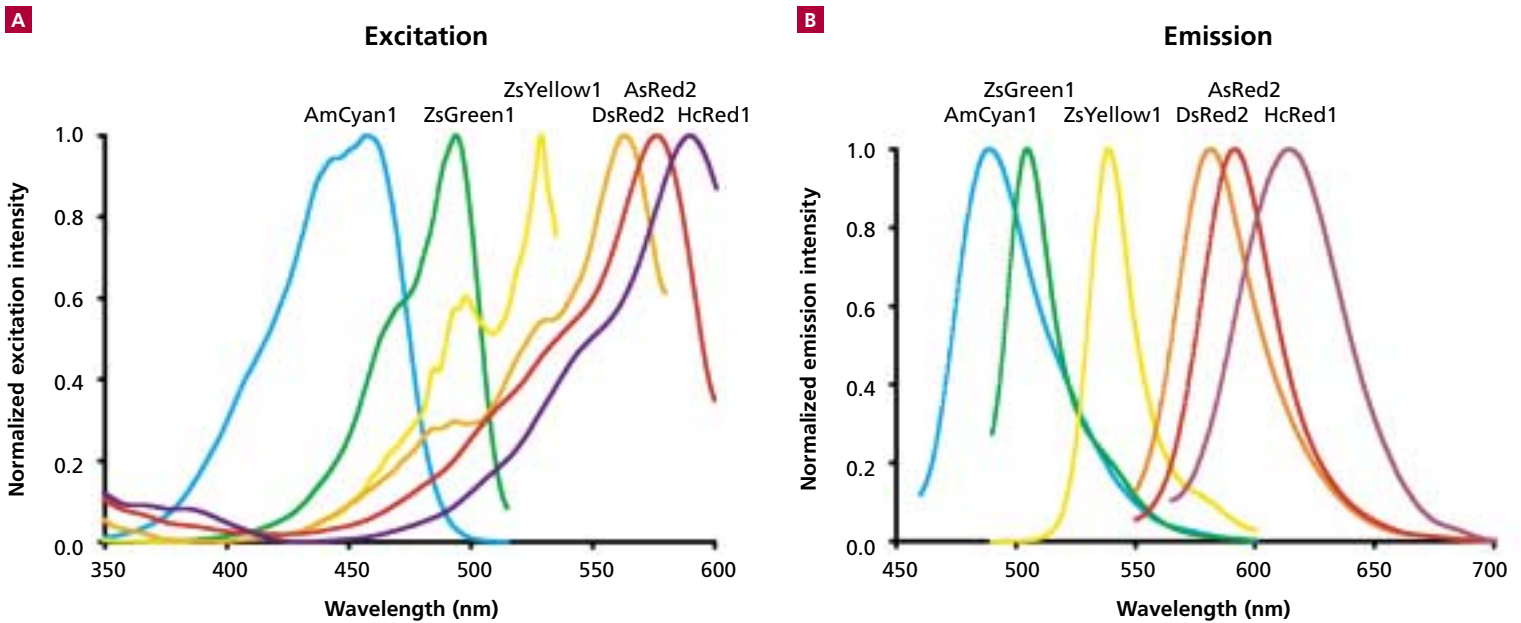


Figure 6. Excitation and emission spectra of BD Living Colors™ Reef Coral Fluorescent Proteins. Panel A: Excitation spectra. Panel B: Emission spectra. The spectra for DsRed-Express (not shown) closely resemble those of DsRed2. As compared to DsRed2, however, DsRed-Express has a much lower level of residual green emission.

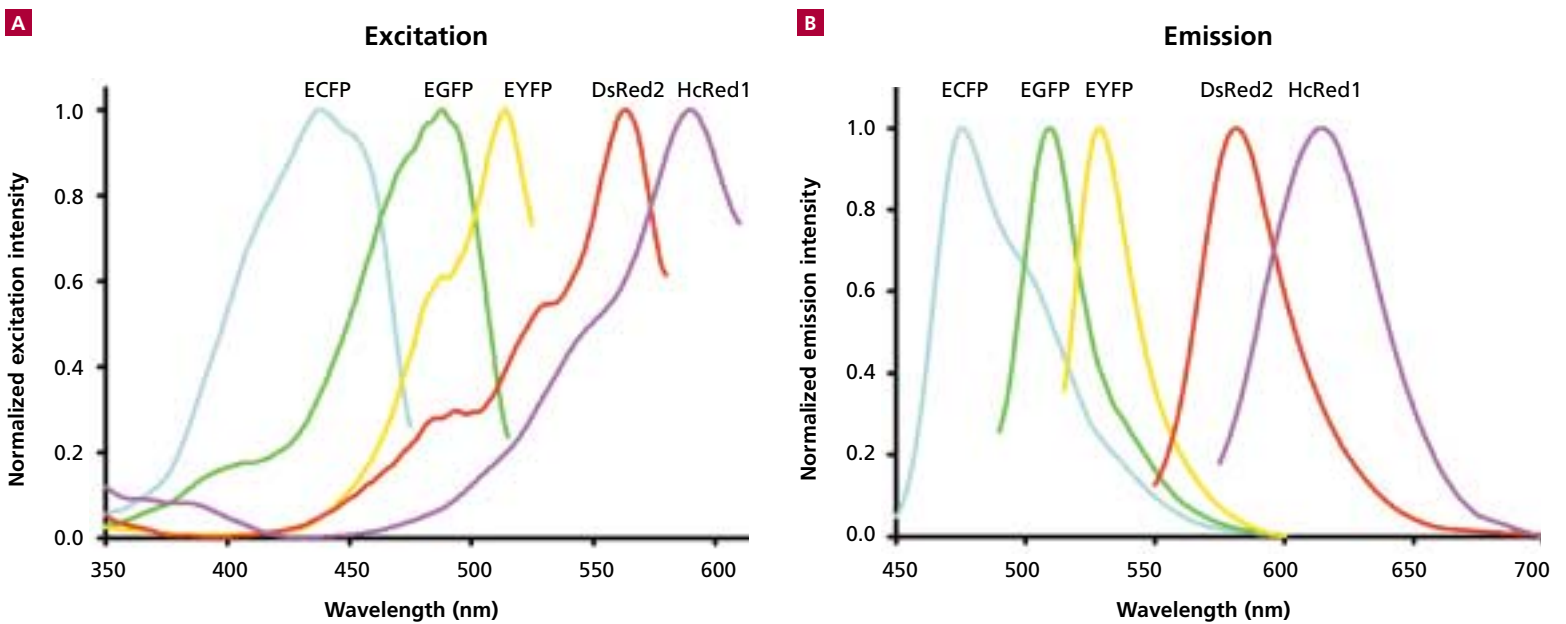


Figure 7. Excitation and emission spectra of BD Living Colors™ *Aequorea victoria* GFP variants. Spectra for DsRed2 and HcRed1 are also shown. Panel A: Excitation spectra. Panel B: Emission spectra.

BD Living Colors™ Vectors

Suitable for a wide range of applications

- Carry out gene expression studies, monitor promoter activity, and study protein localization in living cells
- Develop cell-based assays
- Create transgenic organisms

We offer a complete line of vectors for expressing fluorescent proteins in both bacterial and mammalian cell systems. Our bacterial vectors are designed with flanking multiple cloning sites (MCS) so that you can easily excise the cDNA for use in other constructs. BD Living Colors proteins can be expressed and detected in a wide range of organisms, including bacteria, yeast, *C. elegans*, *Drosophila*, *Xenopus*, Zebrafish, mice, human cells, and plants.

Study protein localization in living cells

An easy way to track a protein's location in the cell is to express the protein as a fluorescent fusion, constructed with one of our N- or C-terminal fusion vectors. Protein translocation, for example, as occurs during the activation of certain biochemical pathways, such as signal transduction and apoptosis, can be directly visualized if the protein involved is tagged with a BD Living Colors protein (Figures 8 & 9). Unlike antibody and dye-based detection methods, the analysis does not require fixation or multiple washing steps, so you can perform many cell-based assays in very little time—perfect for drug screening applications.



Figure 8. Activation of PKC α detected with HcRed1. HEK 293 cells were transiently transfected with pPKC α -HcRed1, a recombinant vector that encodes protein kinase C (PKC α) as a fusion with HcRed1. The next day, cells were treated with 1.5 μ g/ml phorbol 12-myristate 13-acetate (PMA) to activate PKC, and then visualized by fluorescence microscopy. **Panel A:** Before treatment. **Panel B:** After treatment, the HcRed1-PKC α fusion visibly translocated from the cytosol to the plasma membrane—a result that is consistent with PKC α 's known translocation pattern upon activation.

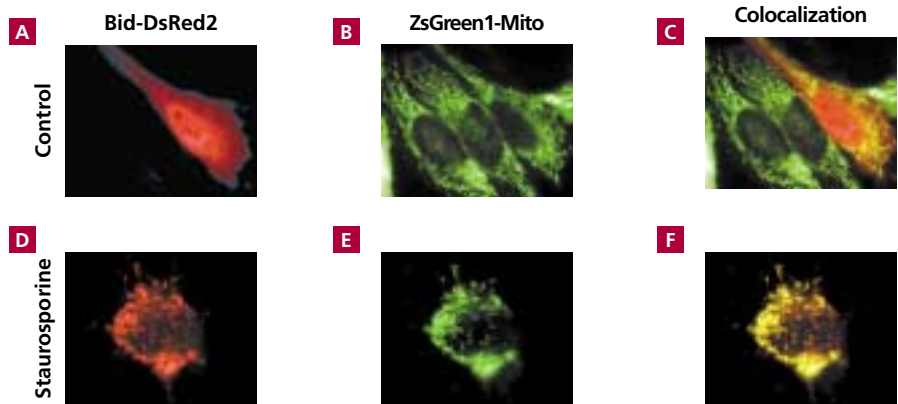


Figure 9. Monitoring Bid activation with DsRed2. HeLa cells were transiently cotransfected with plasmids encoding the fusion protein Bid-DsRed2 and a mitochondrial-targeted ZsGreen1 (ZsGreen1-Mito). **Panels A–C:** before induction of apoptosis; **Panels D–F:** after induction of apoptosis with 1 μ M staurosporine for 3 hours. **Panel A:** In healthy cells, Bid-DsRed2 is localized in the cytosol; **Panel B:** ZsGreen1-Mito labels the mitochondria; **Panel C:** overlay of images A and B reveals separate cellular localization of Bid-DsRed2 and ZsGreen1-Mito. After induction of apoptosis, Bid translocates to mitochondria; **Panel D:** Bid-DsRed2; **Panel E:** ZsGreen1-Mito; **Panel F:** overlay of images D and E shows the relocation of Bid-DsRed2 to mitochondria as revealed by the colocalization with the mitochondrial marker ZsGreen1-Mito.

Recombinant Purified Fluorescent Proteins

In addition to vectors, we also offer purified recombinant red fluorescent and green fluorescent proteins for use as positive controls and as standards in studies involving their expression *in vivo* and *in vitro*. They can be used as standards in fluorometric studies or as controls in polyacrylamide gels and Western blots. They are well tolerated by mammalian cells and should be suitable for use in microinjection studies as well.

BD Living Colors™ Vectors...continued

Monitor changes in promoter activity

Our promoterless vectors enable you to detect transient changes in promoter activity. These vectors contain a single MCS, located just upstream of the fluorescent protein coding sequence. A *cis*-acting regulatory element inserted into the MCS will be functionally linked to the gene, allowing you to measure promoter activation at different time points and under different conditions. Many of our promoterless vectors encode destabilized variants—fluorescent proteins that are engineered for rapid turnover. When placed under the control of an inducible promoter, destabilized variants exhibit a higher fold-induction upon activation. That's because the small amount of protein expressed in the uninduced state is rapidly degraded. With our destabilized variants, you can measure both the up- and down-regulation of promoter activity (Figure 10).

Monitor protein turnover

The Fluorescent Timer protein, encoded by our pTimer and pTimer-1 vectors, enables you to see not only *where* a promoter becomes active but also *when* it becomes inactive. A variant of DsRed, Fluorescent Timer undergoes a predictable color shift from green to red within several hours of its expression *in vivo* (4). Green fluorescence indicates recently translated material, whereas red denotes matured protein. By monitoring the ratio of green to red fluorescence you can detect the onset and termination of promoter activity (Figure 11), protein turnover rates in cells, cell lineages in whole organisms, cycling events, and even bulk protein production.

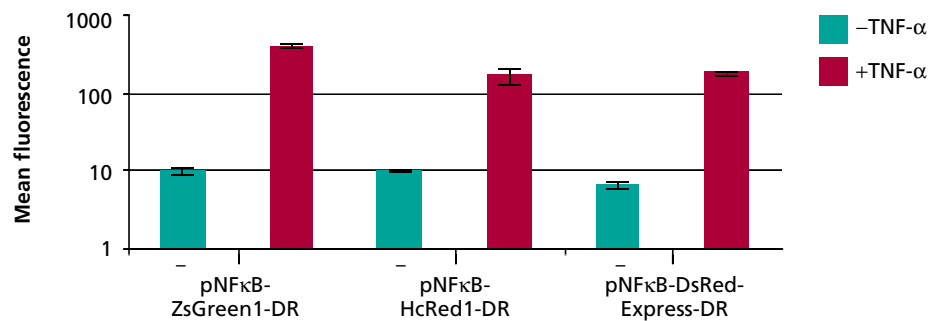


Figure 10. Detect the onset of promoter activity with promoterless vectors encoding destabilized fluorescent protein variants. To measure the activation of NFκB—a transcription factor known to regulate several genes involved in inflammation, immune response, and apoptosis—the NFκB DNA response element was cloned into the MCS upstream of the fluorescent reporter gene in the indicated vector. The constructs were then transiently transfected into HeLa cells. After overnight incubation, cells were analyzed using a BD FACS Vantage™ flow cytometry system at two 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. Please note that the data are plotted on a logarithmic scale.

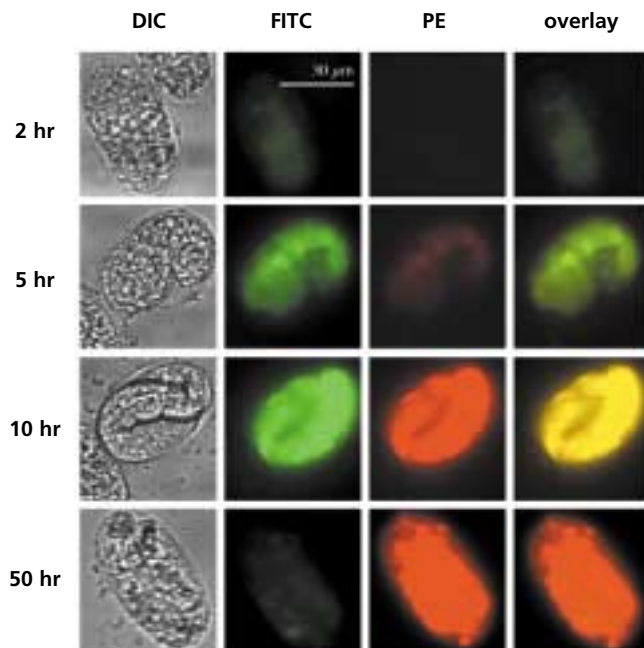


Figure 11. Fluorescent Timer lets you visualize changes in promoter activity. A transgenic *C. elegans* embryo containing Fluorescent Timer under the control of the heat shock promoter hsp16-41 was heat-shocked by flotation in a 33°C water bath. Promoter activity was studied during the heat shock recovery period. If both green and red fluorescence are present, the two colors together appear yellow in an overlay.

BD Living Colors™ Subcellular Localization Vectors

Visualize specific organelles or structures in living cells

BD Living Colors™ Subcellular Localization Vectors allow you to visualize subcellular structures directly and noninvasively by fluorescence microscopy. Members of this vector family encode fusions of fluorescent protein variants to localization signals or subcellular structural proteins, which target the fluorescent protein to a specific organelle or subcellular structure. The vectors are available in a variety of organelle- and cytoskeleton-targeted color variants (Figure 12 and Table II). You can monitor the location of a protein of interest relative to a given subcellular structure by labeling both the protein and the structure with separate fluorescent proteins.

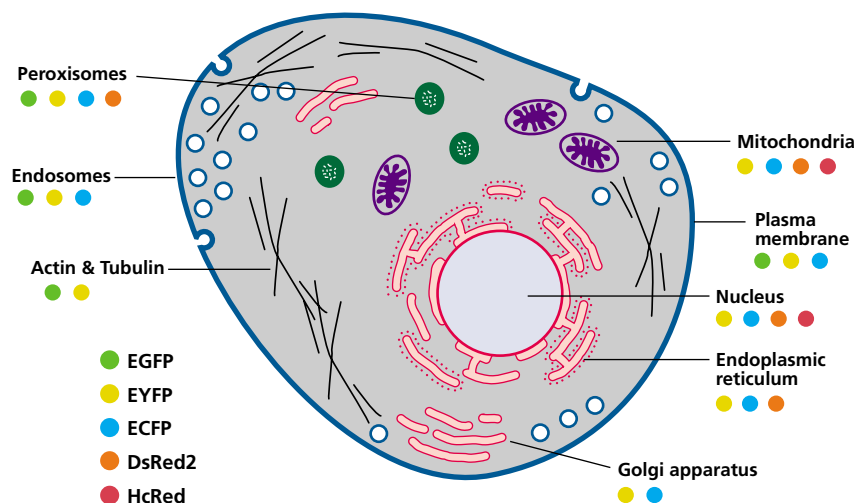


Figure 12. Organelles targeted by BD Living Colors™ Subcellular Localization Vectors.

Table II: BD Living Colors™ Subcellular Localization Vectors

Targeted subcellular structure	Color variants available	Localization tag or gene	Potential applications
Endosomes	Green, cyan, yellow	RhoB	<ul style="list-style-type: none"> Observe movement of vesicles of endocytic pathway Monitor endocytosis of labeled receptors or ligands
Mitochondria	Cyan, yellow, red, far red	Targeting sequence from subunit VIII of cytochrome c oxidase	<ul style="list-style-type: none"> Study normal & disease state Track mitochondrial dynamics
Nucleus	Cyan, yellow, red, far red	SV40 T-antigen NLS; 3 tandem repeats	<ul style="list-style-type: none"> Study nuclear import Track cell lineage Monitor cell growth & division
Endoplasmic reticulum	Cyan, yellow, red	Targeting sequence of calreticulin; KDEL retrieval sequence	<ul style="list-style-type: none"> Visualize tubules & cisternae Track morphology & intracellular distribution
Golgi apparatus	Cyan, yellow	Targeting sequence from human β 1,4-galactosyltransferase	<ul style="list-style-type: none"> Study organelle dynamics Track morphology & intracellular distribution
Plasma membrane	Green, cyan, yellow	Palmitoylation domain of neuromodulin; farnesylation sequence from Ha-Ras (pEGFP-F)	<ul style="list-style-type: none"> Study membrane dynamics & protrusions Monitor membrane-associated changes during apoptosis
Peroxisome	Green, cyan, yellow, red	Peroxisomal targeting signal 1 (PST1)	<ul style="list-style-type: none"> Monitor movement, segregation, biogenesis & degradation Study peroxisome purification
Actin filaments	Green, yellow	Human β -actin dynamics	<ul style="list-style-type: none"> Study cytoskeletal dynamics Monitor co-localization with associated proteins or organelles
Microtubules	Green, yellow	Human α -tubulin	<ul style="list-style-type: none"> Study cytoskeletal dynamics Monitor co-localization with associated proteins or organelles

IRES Bicistronic Expression Vectors

Quickly identify cells expressing your protein of interest

- Save time developing functional stable cell lines
- Eliminate the necessity of clonal selection
- Select for stable clones with high-level protein expression

IRES Bicistronic Expression Vectors allow you to rapidly and efficiently select positive clones that express your target protein. They include a single cassette that expresses both your gene of interest and a BD Living Colors fluorescent protein from the same promoter (Figure 13) so that virtually all transfected cells expressing the selection marker also express your gene of interest. With these vectors, you can screen fewer colonies to locate clones that are expressing high levels of your protein.

The pIRES Vectors contain the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA (1, 2). Ribosomes can enter the bicistronic mRNA either at the 5' end to translate the gene of interest or at the ECMV IRES to translate the selection marker or reporter gene (Figure 14).

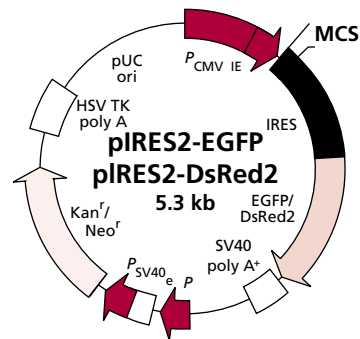


Figure 13. Composite vector map for BD Living Colors™ IRES vectors. In a third vector, pLP-IRES2-EGFP, the MCS is replaced with a *LoxP* site to make it compatible with the BD Creator™ Gene Cloning and Expression System.

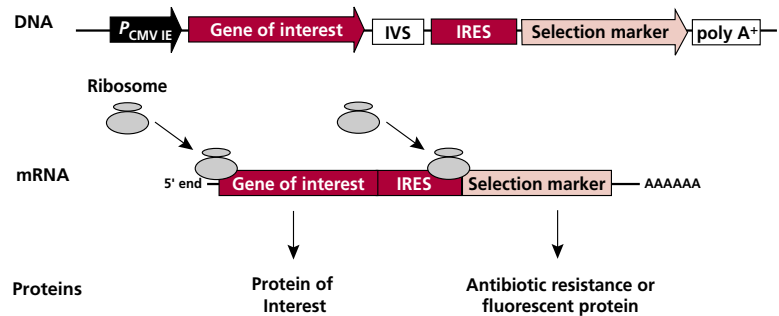


Figure 14. Schematic diagram of the translation of a bicistronic mRNA. The internal ribosome entry site (IRES) permits a protein of interest and an antibiotic or fluorescent protein selection marker to be translated from the same mRNA. IVS = synthetic intron.

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Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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Antibodies

For the detection and quantification of BD Living Colors™ Fluorescent Proteins

- **Highly specific monoclonal and polyclonal antibodies—compatible with a wide range of applications**
- **Purified recombinant green and red fluorescent proteins**

BD Biosciences Clontech offers a wide variety of monoclonal and polyclonal antibodies for the detection of BD Living Colors Fluorescent Proteins by Western blotting, immunoprecipitation, or cytochemical assay (Table III). The antibodies are especially useful for confirming N- and C-terminal fusions following their expression *in vivo* (Figures 15 and 16). Because Reef Coral Fluorescent Proteins and *Aequorea victoria* GFP variants are derived from different organisms, the antibodies for these two families display minimal cross reactivity. Furthermore, because each RCFP is a unique protein encoded by a distinct gene rather than a mutant variant of a single fluorescent protein (as in the *A. victoria* family), it has been possible to develop highly specific antibodies against certain individual RCFPs (Table III).

References

1. Yang, T. T. *et al.* (1996) *Nucleic Acids Res.* 24:4592–4593.
2. Patterson, G. H. *et al.* (1997) *Biophys. J.* 73:2782–2790.
3. Hawley, T. S. *et al.* (2001) *Biotechniques* 30:1028–1034.
4. Terskikh, A. *et al.* (2000) *Science* 290:1585–1588.

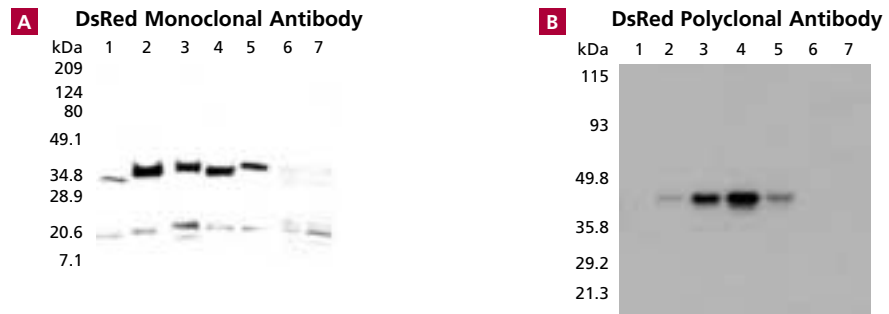


Figure 15. Western blot detection of DsRed and its variants. HeLa cells were transiently transfected with the following vectors, and then analyzed by Western blot. **Panel A.** Western blot with the DsRed Monoclonal Antibody. Lane 1: pDsRed2-N1, Lane 2: pDsRed2-C1, Lane 3: pDsRed1-C1, Lane 4: pDsRed2-Mito (N-terminal fusion), Lane 5: pDsRed2-Nuc (C-terminal fusion), Lane 6: pEGFP-N1, Lane 7: Control (untransfected cells). A 20 kDa, non-specific band appears with extended exposure. **Panel B.** Western blot with the DsRed Polyclonal Antibody. Lane 1: control (untransfected cells), Lane 2: pDsRed1-N1, Lane 3: pDsRed2-N1, Lane 4: pCMV-DsRed-Express, Lane 5: pTimer, Lane 6: pHcRed1-N1, Lane 7: pEGFP-N1. The DsRed Polyclonal Antibody was used at a 1:16,000 dilution. The weak signal in Lane 2 is due to the low abundance of DsRed1 in the cells.



Figure 16. Western blot detection of GFP and its variants. **Panel A.** Western blot using the A.v. Peptide Antibody at a 1:400 dilution. **Panel B.** Western blot using the A.v. Monoclonal Antibody (JL-8) at a 1:8,000 dilution. Lane 1: Recombinant GFP (15 ng); Lane 2: Recombinant EGFP (50 ng); Lanes 3–5: Lysates from HeLa cells expressing either a negative control (Lane 3), an EGFP fusion protein (Lane 4), or EYFP-Mito (Lane 5)—a fluorescent fusion protein that contains a mitochondrial targeting sequence.

Table III: BD Living Colors™ Antibodies

Antibody Name	Cat. No.	Antibody Characteristics	Suitability for Applications		
			Western Blot	Immuno-precipitation	Immuno-cytochemistry
A.v. Monoclonal Antibody (JL-8)	632380	Affinity-purified mouse monoclonal (IgG2a)	+++	++	+++
Full-Length A.v. Polyclonal Antibody	632382	Rabbit polyclonal serum, generated using full length GFP protein	++	+++	not recommended
A.v. Peptide Antibody (polyclonal)	632377	Rabbit polyclonal, mixture of three affinity-purified peptide Ab's to GFP	+++	++	+++
GFP Monoclonal Antibody	632375	Affinity-purified mouse monoclonal	+++	not recommended	+
DsRed Monoclonal Antibody	632393	Mouse monoclonal recognizes DsRed and its variants	+++	not determined	not determined
DsRed Polyclonal Antibody	632397	Rabbit polyclonal serum recognizes DsRed and its variants	+++	++	+++
HcRed Polyclonal Antibody	632452	Rabbit polyclonal recognizes HcRed1 as well as N- and C- terminal fusions to HcRed1	+++	not recommended	not recommended

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