BD Living Colors™ AcGFP1 Fluorescent Protein

A novel monomeric green fluorescent protein alternative to EGFP

- **Monomeric green fluorescent protein, excellent for fusion tag applications**
- **Ideal for dual labeling with all DsRed variants**
- **Equivalent in brightness and performance to EGFP**
- **Fully compatible with existing filter sets and BD Living Colors™ Antibodies**

BD Living Colors™ AcGFP1 Vectors encode a distinct green fluorescent protein that has been engineered to provide excellent performance when expressed as a fusion with your protein of interest. It's ideal for multicolor applications in flow cytometry (such as BD FACS™ analysis) and fluorescence microscopy.

AcGFP1 was derived from the jellyfish *Aequorea coerulescens* and is a novel alternative to monomeric enhanced GFP (EGFP) (1). AcGFP1 is an engineered fluorescent mutant of the wild-type protein with 94% homology to EGFP at the amino acid level. It features an open reading frame that has been human codon optimized. This optimization increases the translational efficiency of the AcGFP1 mRNA and results in higher mammalian cell expression levels.

The AcGFP1 protein is stable, allowing you to monitor fluorescence over extended periods of time. The chromophore matures rapidly and is readily detected in 8–12 hr. Like all BD Living Colors Novel Fluorescent Proteins (NFPs), AcGFP1 can be detected in cells without adding cofactors or substrates (2–4).

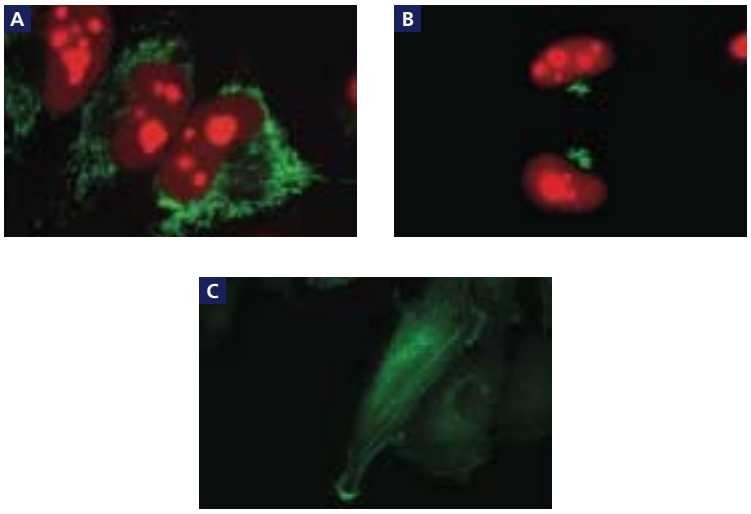

Figure 1. BD Living Colors™ AcGFP1 is ideal for multicolor and fluorescence microscopy applications. AcGFP1 and DsRed2 protein fusions were transiently transfected and visualized by fluorescence microscopy. **Panel A.** pAcGFP1-Mito (mitochondria) and pDsRed2-Nuc (nucleus) in HEK 293 cells. **Panel B.** pAcGFP1-Golgi (Golgi apparatus) and pDsRed2-Nuc (nucleus) in HEK 293 cells. **Panel C.** pAcGFP1-Actin (actin) in HeLa cells.

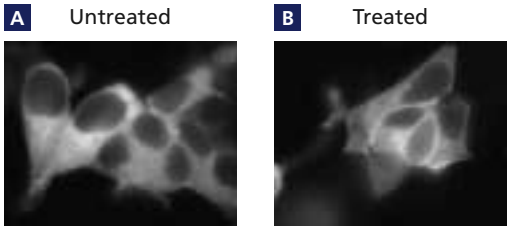

Figure 2. Activation of Protein Kinase C α (PKCα) can be monitored with AcGFP1. HEK 293 cells were stably transfected with a plasmid encoding AcGFP1 fused to PKCα. Cells were induced with 1.5 µg/ml PMA for 3 min. The PKCα-AcGFP1 fusion moves from the cytosol to the plasma membrane—a result consistent with the known mobilization pattern of PKCα.

AcGFP1 protein is an ideal fluorescent tag for visualizing and tracking a protein of interest. The use of AcGFP1 as a fusion tag has been validated by several methods using a wide variety of proteins with diverse functions and subcellular locations. With excitation and emission spectra distinct from those of DsRed proteins (such as DsRed2 and DsRed-Express), AcGFP1 is particularly suited for use in multicolor applications with DsRed to simultaneously visualize the subcellular localization of two proteins of interest (***Figure 1***). It also performs well in cell-based assays monitoring protein subcellular trafficking (***Figure 2***).

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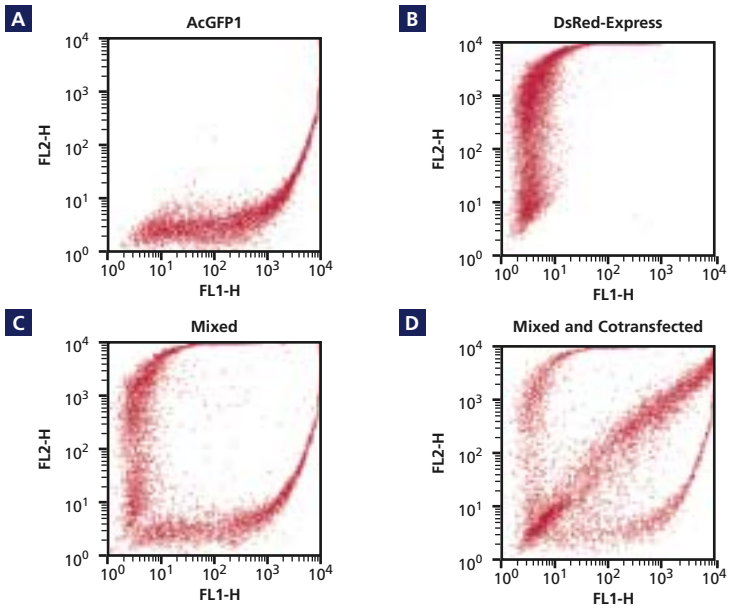

Figure 3. Cells expressing AcGFP1 protein are easily detected by standard BD FACS™ analysis. HEK 293 cells were transfected with the construct indicated and analyzed with the BD FACSVantage™ SE Flow Cytometry System 24 hr post-transfection. **Panel A.** pAcGFP1 alone. **Panel B.** pDsRed-Express alone. **Panel C.** Cells were separately transfected with either pAcGFP1 or pDsRed-Express. The two cell populations were mixed prior to analysis. **Panel D.** Cells were cotransfected with constructs expressing pAcGFP1 and pDsRed-Express. The mixture from Panel C was added to this cotransfected population prior to analysis. The cotransfected population appears along the diagonal.

In addition, AcGFP1 is easily detected and sorted by flow cytometry (***Figure 3***). As with all of our NFPs, AcGFP1 is well tolerated by mammalian cells, and has been successfully used to establish stably transfected, clonal cell lines.

A true monomer

The monomeric nature of AcGFP1 protein has been confirmed by three independent methods. When recombinant AcGFP1 is analyzed by FPLC gel filtration chromatography, it elutes in a single uniform peak at a retention time consistent with a molecular weight of 24 kDa (***Figure 4, Panel A***). Further analysis by sucrose density gradient ultracentrifugation yields a profile both consistent with a monomeric protein and similar to EGFP (***Figure 4, Panel B***). Pseudonative gel electrophoresis of recombinant AcGFP1 protein in comparison to EGFP (monomeric) and DsRed-Express (oligomeric) also substantiates this conclusion (***Figure 4, Panel C***). All of these results agree with the calculated molecular weight of 26.9 kDa based on amino acid sequence.

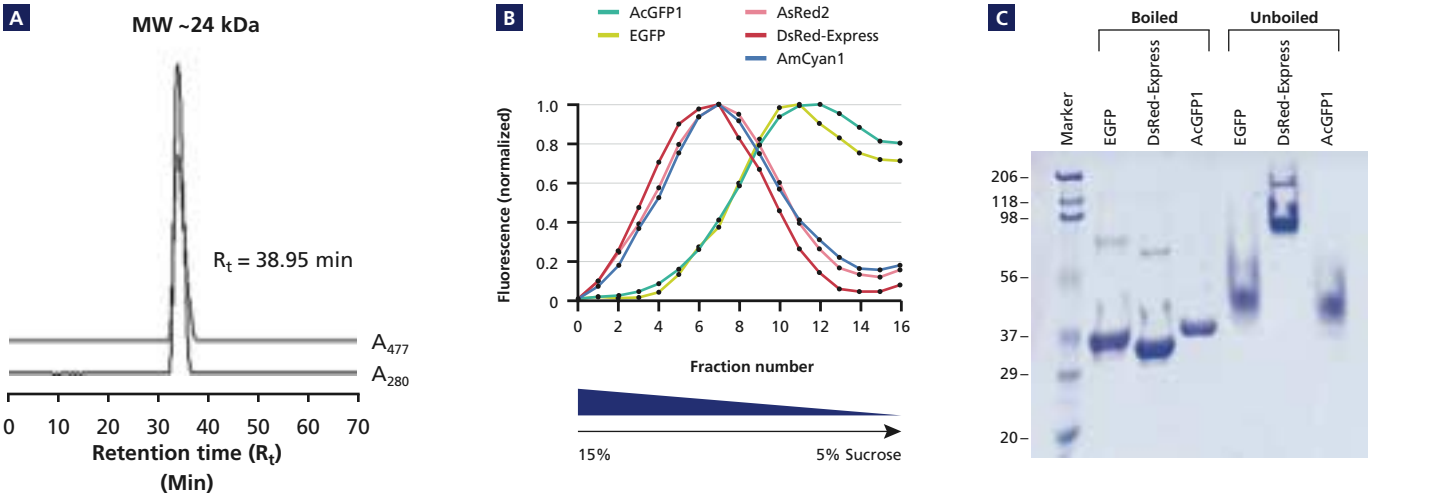

Figure 4. AcGFP1 is a monomeric protein. Panel A. Recombinant AcGFP1 protein was analyzed by FPLC gel filtration chromatography. Overall protein absorption (A_{280}) and chromophore excitation (A_{477}) of the eluted material was monitored simultaneously. AcGFP1 elutes from the column at a retention time corresponding to a molecular weight of 24 kDa. The calculated molecular weight of AcGFP1 is 26.9 kDa. **Panel B.** Recombinant AcGFP1 protein was analyzed by sucrose density ultracentrifugation using a continuous gradient. The fractionation profile of AcGFP1 follows that of EGFP. **Panel C.** Pseudonative gel analysis of proteins. The oligomeric structure of proteins is 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, EGFP and AcGFP1 run as uniform bands of ~30 kDa due to their monomeric structure. The unboiled (undenatured) DsRed-Express runs at a much higher molecular weight than its denatured (boiled) counterpart due to its oligomeric structure.

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Table I: Spectral properties of AcGFP1 protein

Protein	Excitation Max (nm)	Emission Max (nm)	Time to detection (hr)	Relative fluorescent intensity	Quaternary structure	Utility as a reporter	Utility in fusions	Extinction Coefficient ($M^{-1}cm^{-1}$)	Quantum Yield
AcGFP1	475	505	8–12	Bright	Monomer	Good	Excellent	32,500	0.82
EGFP	484	510	8–12	Bright	Monomer	Good	Excellent	23,000	0.70
ZsGreen	493	505	8–12	Extremely Bright	Tetramer	Excellent	Fair	43,000	0.91

Compatible with existing filter sets and BD Living Colors™ antibodies

AcGFP1 has an excitation maximum of 475 nm and an emission maximum of 505 nm (***Table I & Figure 5***). Its similar brightness and spectral properties to EGFP allow AcGFP1 to be detected using existing filter sets for EGFP or FITC. This includes standard factory-installed microscope filters as well as custom-tailored, optimized sets such as those available from Chroma Technology Corporation (***Table II***). Furthermore, AcGFP1 can be easily detected in both Western blot and immunoprecipitation applications by our wide array of BD Living Colors Antibodies including the Full-Length A.v. Polyclonal and GFP Monoclonal antibodies (***Table III***).

Wide range of vectors available

We have a large number of AcGFP1 vectors available for multiple applications. Our collection includes source vectors and those that express AcGFP1 as either an N- or C-terminal fusion, or as a reporter (with a bicistronic internal ribosome entry sequence, IRES2). In addition, a wide range of subcellular localization vectors, including Actin and Golgi markers, are available. BD Creator™ Acceptor vectors featuring AcGFP1 allow both N- and C-terminal cloning of AcGFP1 into our versatile BD Creator™ system (5). Our wide range of vectors provide you with an excellent set of tools to address your diverse application needs.

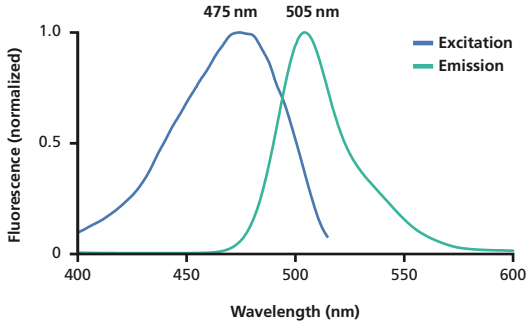

Figure 5. Fluorescent excitation and emission spectra of AcGFP1.

Table II: Filter sets for AcGFP1 detection

Description	Features
EGFP/AcGFP1 Chroma No. 32000	Exciter D425/60x Dichroic 470DCXR Emitter D510/40m
EGFP/AcGFP1 Preferred Set Chroma No. 41017	Exciter HQ470/40x Dichroic Q495LP Emitter HQ525/50m

Table III: BD Living Colors™ Antibodies for AcGFP1

Antibody name	Cat. No.	Antibody characteristics	Application	
			Western	IP
A.v. Monoclonal Antibody (JL-8)	632380 632381	Affinity-purified mouse monoclonal (IgG2a)	+++	Not determined
Full-Length A.v. Polyclonal Antibody	632359 632360	Rabbit polyclonal serum generated using full-length GFP protein	+++	+++
A.v. Peptide Antibody (Polyclonal)	632376 632377	Rabbit polyclonal; mixture of three affinity-purified antibodies to GFP	++	++
GFP Monoclonal Antibody	632375	Affinity-purified mouse monoclonal	+++	Not determined

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

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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**.

BD Living Colors™ Protein Licensing Program

AcGFP1 is just one of the many fluorescent proteins exclusively available to commercial companies through our Licensing Program. The program includes AcGFP, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow, and their variants. For further information, please contact our licensing hot line at 800.424.8222, ext. 7816 or e-mail us at licensing@clontech.com.

References

1. Gurskaya, N. G. *et. al.*, (2003) *Biochem. J.* **373**:403–408.
2. Reef Coral Fluorescent Protein Vectors (July 2003) *Clontechniques* **XVIII**(3):6–7.
3. Matz, M. V., *et al.* (1999) *Nature Biotechnol.* **17**:969–973. Erratum in: *Nature Biotechnol.* (1999)**17**:1227.
4. BD Living Colors™ DsRed-Express (July 2003) *Clontechniques* **XVII**(3):16.
5. Creator™ System Overview (October 2001) *Clontechniques* **XVI**(4):5–6.

Product List

BD Living Colors™ AcGFP1 Products

PRODUCT	SIZE	CAT. NO.
pAcGFP1 Vector	20 µg	632468
pAcGFP1-C1 Vector	20 µg	632470
pAcGFP1-N1 Vector	20 µg	632469
AcGFP Vector Set	3 x 20 µg	632426
pIRES2-AcGFP1 Vector	20 µg	632435
pAcGFP1-Actin Vector	20 µg	632453
pAcGFP1-Golgi Vector	20 µg	632464
pAcGFP1-Mito Vector	20 µg	632432
pAcGFP1-Nuc Vector	20 µg	632431
pLP-AcGFP1-C Acceptor Vector	20 µg	632471
pLPS-AcGFP1-N Acceptor Vector	20 µg	632472

Related Products

- A.v. Monoclonal Antibody (JL-8) (Cat. Nos. 632380 & 632381)
- Full-Length A.v. Polyclonal Antibody (Cat. Nos. 632359 & 632360)
- GFP Monoclonal Antibody (Cat. No. 632375)
- A.v. Peptide Antibody (Polyclonal) (Cat. Nos. 632377 & 632376)

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