Green-to-red photoswitchable fluorescent protein Dendra2

- Monomer, successful performance in fusions
- Irreversible photoconversion from a green to a red fluorescent form
- High contrast of photoconversion
- Activated by UV-violet and blue light
- Matures at a wide range of temperatures
- Recommended for tracking cell, organelle, and protein movement, and for determination of protein half-life

Description

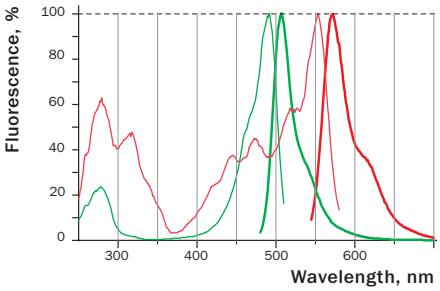
Dendra2 is an improved version of a green-to-red photoswitchable fluorescent protein Dendra, derived from octocoral *Dendronephthya* sp. (Gurskaya *et al*., 2006). Dendra2 exhibits faster maturation and brighter fluorescence both before and after photoswitching than that of Dendra.

Dendra2 is capable of irreversible photoconversion from a green to a red fluorescent form. Comparing with other available photoactivatable proteins, it provides a unique combination of advantageous properties including monomeric state suitable for protein labeling, high contrast photoconversion with fluorescence at the red spectral region, low-phototoxic activation with 488-nm light available on common confocal microscopes, high photostability of the photoconverted state, and efficient chromophore maturation at 37°C in mammalian cells. These properties make Dendra2 an ideal tool for real-time tracking protein dynamics (movement, degradation, etc.) and monitoring selective cell fate (Gurskaya *et al*., 2006; Zhang *et al*., 2007; Chudakov *et al*., 2007).

Main properties of Dendra2

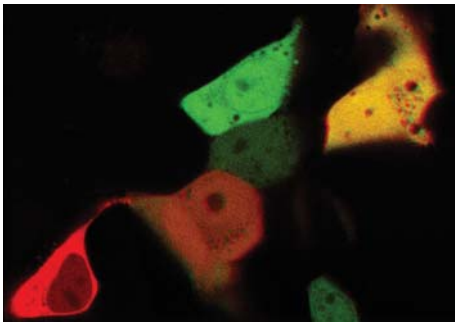
Characteristic		
Molecular weight	26 kDa	
Polypeptide length	230 aa	
Structure	monomer	
Aggregation	no	
Maturation rate at 37°C	fast	
Activating light	UV-violet (e.g. 405 nm) or blue (e.g. 488 nm)	
Contrast, fold	up to 4000	
	before photoconversion	**after photoconversion**
Fluorescence color	green	red
Excitation max	490 nm	553 nm
Emission max	507 nm	573 nm
Quantum yield	0.50	0.55
Extinction coefficient, $M^{-1}cm^{-1}$	45 000	35 000
Brightness*	22.5	19.3
pKa	6.6	6.9

*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.



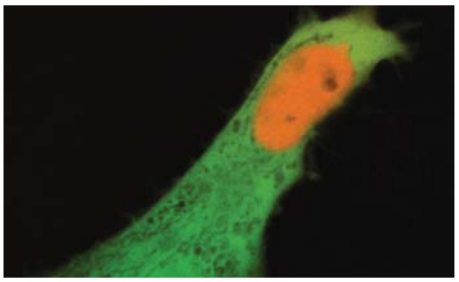
Normalized excitation (thin lines) and emission (thick lines) spectra for non-activated (green lines) and activated (red lines) Dendra2.

Dendra2 spectra in Excel format can be downloaded from the Evrogen Web site at www.evrogen.com/ Dendra2.shtml.



Cell photolabeling with green-to-red photoconvertible fluorescent protein Dendra2.

HEK293 Phoenix Eco cells were transiently transfected with Dendra2 gene under the control of CMV promoter. Dendra2 was converted to the red state in selected cells by brief illumination with 405-nm (left cell) or 488-nm (upper right and middle cells) lasers. Then confocal images of cells were made in green and red channels and overlaid.



Green-to-red photoconversion of Dendra2 in cell nucleus.

HeLa cells were transiently transfected with Dendra2 gene under the control of CMV promoter. Dendra2 was converted in a nucleus by brief illumination with 405-nm laser. Then confocal images of a cell were made in green and red channels and overlaid.

Available variants and fusions

Dendra is a mutant of the GFP-like protein from octocoral *Dendronephthya* sp. (Gurskaya *et al.*, 2006). Compared with Dendra, Dendra2 comprises single A224V substitution, which results in better maturation and a brighter fluorescence both before and after photoswitching.

Dendra2 codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996), but it can be successfully expressed in many other heterological systems.

Dendra2-At variant

Dendra2-At codon usage is optimized for high expression in *Arabidopsis*. This variant is available in Gateway® entry clones.

Performance and use

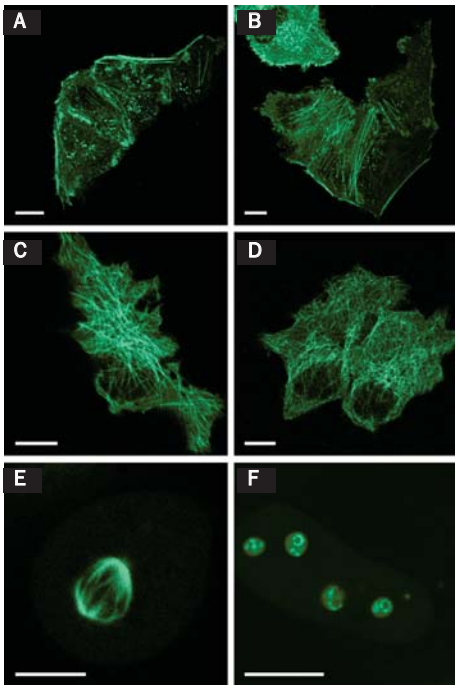
Dendra2 efficiently matures both at 20°C and 37°C, which makes possible the use of the protein in wide range of experimental systems, from cultured mammalian cells to cold-blooded animals. Mammalian cells transiently transfected with Dendra2 expression vectors display an evenly distributed green signal without aggregation within 10-12 hrs after transfection. No cell toxicity is observed. High photostability of photoconverted Dendra2 (more than 3 times higher than of DsRed) makes it particularly useful for long-term protein tracking applications.

Dendra2 successful performance has been proven in many fusions including that with cytoplasmic beta-actin, BH3 interacting domain death agonist (BID), nucleolar protein fibrillarin, vimentin, and alpha-tubulin.

High contrast of photoconversion: In response to intense 405 nm or 488 nm light irradiation, Dendra2 undergoes irreversible photoconversion expressed in a decrease in green and appearance of red fluorescence. After complete photoconversion, red fluorescence of Dendra2 increases more than 150-300 times, whereas the level of green fluorescence becomes more than 10-15 times lower. Thus, the increase in the red-to-green fluorescence ratio results in about a 4000-fold contrast. Considerable decrease of green fluorescence during Dendra2 photoconversion provides a molecular tool to simultaneously track both the movement of the activated protein and its replacement with the non-activated form.

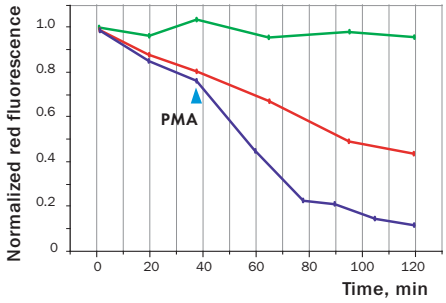
Dendra2 use for determination of protein half-life: In the method proposed, cells are transfected with a construct coding for target protein fused with a photoswitchable tag (PAFP). A steady-state concentration of the fusion protein and corresponding fluorescent signal depends on protein synthesis and maturation rates as well as protein degradation rate. After photoconversion of the photoswitchable tag in a whole cell, a pool of distinct fluorescent molecules appears, which is independent of the synthesis and maturation of the new PAFP molecules. Thus, the decay of the activated fluorescence directly corresponds to the degradation of the PAFP-tagged protein. Time-lapse imaging of the activated signal allows for quantification of degradation process in real-time at the single cell level (Zhang *et al.*, 2007).

To test the applicability of Dendra2 for determination of protein half-life, it was fused with IkappaB-alpha protein, having well-characterized decay in cells. Cells with moderate expression levels of IkappaB-alpha-Dendra2 demonstrated the expected, predominantly cytoplasmic, localization of green fluorescence. After photoconversion, time-lapse series showed fast decay of the red signal with a half-life of 1.5-2 hrs. The addition of a proteasome inhibitor immediately terminated red fluorescence decay. Thus, the decrease of red fluorescent signal was caused by proteasomal degradation of the fusion protein. The rate of red signal decay was in good agreement with the available data on the half-life of IkappaB-alpha obtained using cycloheximide chase. It has been shown earlier that the phorbol ester, phorbol 12-myristate 13-acetate (PMA), increases the IkappaB-alpha



Labeling of intracellular proteins with Dendra2.

Confocal images of HeLa cells transiently expressing Dendra2-tagged proteins: A, B — beta-actin; C — vimentin; D, E — alpha-tubulin; F — fibrillarin. Scale bar, 10 µm.



Monitoring protein degradation using Dendra2 photoconversion.

Graphs show time course of red fluorescence change in HEK293 Phoenix Eco cells after Dendra2 photoconversion by blue light. Each line corresponds to a representative individual cell (10-15 cells were measured for each experiment). The cells were transiently transfected with: Dendra2 (green) or IkappaB-alpha-Dendra2 (resting cells — red; cells treated with phorbol 12-myristate 13-acetate (PMA, 0.1 µg/ml) at a time point designated by blue arrow — blue). Dendra2 along demonstrates practically no decay, IkappaB-alpha-Dendra2 has a half-life of 1.5-2 hrs in resting cells and 20 min after stimulation with PMA.

degradation rate. Indeed, a considerable acceleration of red fluorescence decay after cell treatment with PMA was detected using photoactivation of IkappaB-alpha-Dendra2 (Zhang *et al*., 2007).

Recommended antibodies, filter sets, and visualization parameters

Antibodies

Dendra2 can be recognized using Evrogen Anti-Dendra2 antibody (Cat.# AB821-AB822).

Primary Dendra2 visualization

Non-activated Dendra2 possesses excitation-emission maxima at 490 and 507 nm, similarly to EGFP and other green fluorescent proteins. Thus, commonly used fluorescence filter sets for EGFP, FITC, and other green dyes (e.g. Omega Optical QMAX-Green and XF100-2) are ideally suitable for Dendra2 green state.

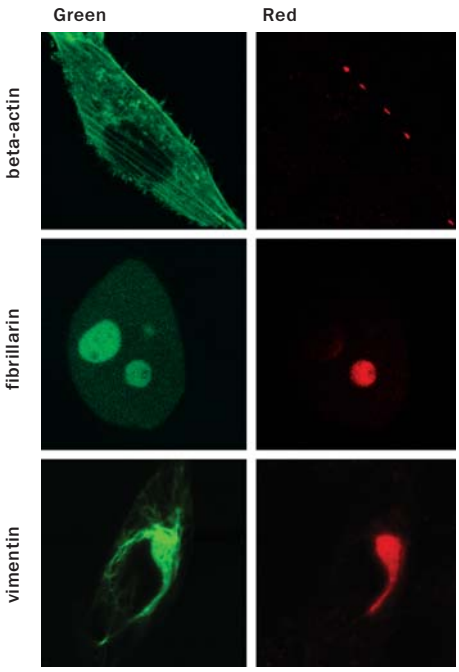
A unique feature of Dendra2 is its photoconversion to red fluorescent state in response to intense-blue-light irradiation at 460-500 nm. In other words, light of the same wavelength is required for both visualization and photoconversion of Dendra2. Importantly, Dendra2 photoconversion occurs only at high light intensities, whereas Dendra2 green fluorescence can be detected at low light intensities. You should carefully select conditions allowing to detect green signal without undesirable photoconversion.

Photoactivation of Dendra2 and Dendra2-tagged proteins

Dendra2 can be photoconverted by light irradiation in either UV-violet (360-420 nm) or blue region (460-500 nm). We recommend that you use 405 nm diode laser or 488 nm Ar laser line. 405-nm laser provides more efficient photoconversion compared with 488-nm laser. However, intense UV-violet light can be harmful for cells.

Tracking Dendra2 and Dendra2-tagged proteins after activation

Activated Dendra2 protein possesses excitation/emission maxima at 553/573 nm. Thus, TRITC filter set or similar (e.g. Omega Optical QMAX-Yellow and XF108-2) can be used for activated Dendra2 visualization. Under the confocal microscope, the red fluorescent signal can be acquired using 543-nm excitation laser line and detected at 560-650 nm.



Green-to-red photoconversion of Dendra2-tagged proteins.
HeLa cells were transiently transfected with vectors encoding Dendra2-tagged fusion proteins which was photoconverted in a selected region by 488-nm laser. Confocal images were made after photoconversion in green and red channels.

References

Chudakov *et al*. (2007) Nat. Protocols 8: 2024-2032..
Gurskaya *et al*. (2006) Nat. Biotechnol. 24(4):461-465.
Haas *et al*. (1996) Curr. Biol. 6: 315–324.
Zhang *et al*. (2007) BioTechniques 42:446-450.

Dendra2-related products

Product	Cat.#	Description	Size
Dendra2 expression/source vectors			
pDendra2-C*	FP821	Mammalian expression vector encoding humanized Dendra2 and allowing Dendra2 expression and generation of fusions to the Dendra2 C-terminus	20 µg
pDendra2-N*	FP822	Mammalian expression vector encoding humanized Dendra2 and allowing Dendra2 expression and generation of fusions to the Dendra2 N-terminus	20 µg
pDendra2-B	FP823	Bacterial expression vector; source of the humanized Dendra2 coding sequence	20 µg
Gateway® Dendra2-At-C	FP824	Gateway® entry clone for generation of fusions to the C-terminus of Arabidopsis-optimized Dendra2; transfer of Dendra2 or its fusion into a Gateway® destination vector for expression in a desired heterological system	20 µg
Gateway® Dendra2-At-N	FP825	Gateway® entry clone for generation of fusions to the N-terminus of Arabidopsis-optimized Dendra2; transfer of Dendra2 or its fusion into a Gateway® destination vector for expression in a desired heterological system	20 µg
Recombinant protein			
rDendra2	FP852	Purified recombinant green-to-red photoswitchable protein	100 µg
Antibodies against Dendra2			
Anti-Dendra2 antibody	AB821 AB822	Rabbit polyclonal antibody against Dendra2	100 µg 200 µg

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