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**

Dendra2 is an improved version of a green-to-red photoswitchable fluorescent protein Dendra, derived 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 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).

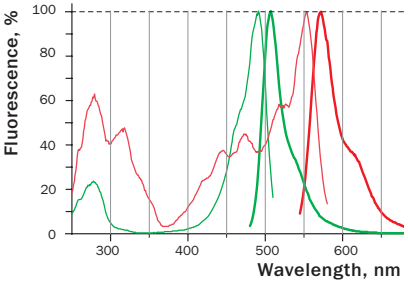
Main properties of Dendra2		
Molecular weight	26 kDa	
Polypeptide length	230 aa	
Structure	monomer	
Aggregation	no	
Maturation rate at 37°C	fast	
Activating light	UV-violet (e.g. 405 nm) or blue (e.g. 488 nm)	
Contrast, fold	up to 4000	
	before photo-conversion	**after photo-conversion**
Fluorescence 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.

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



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

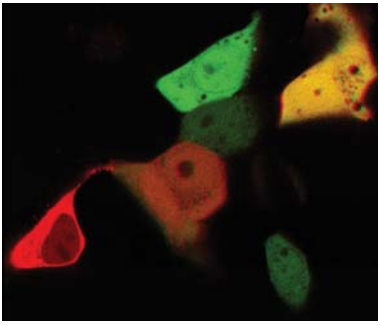
Dendra2 spectra in Excel format can be downloaded at www.evrogen.com/Dendra2.shtml.

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.

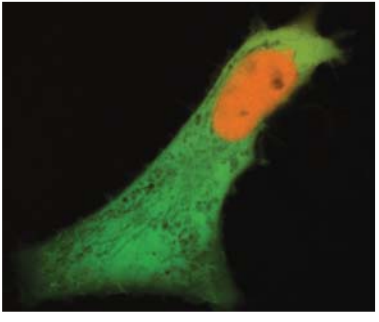
High contrast of photoconversion: In response to intense 405 nm or 488 nm light irradiation, Dendra2 undergoes irreversible photoconversion expressed in a decrease in green and appearance of red fluorescence. After complete photoconversion, red fluorescence of Dendra2 increases more than 150-300 times, 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.

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

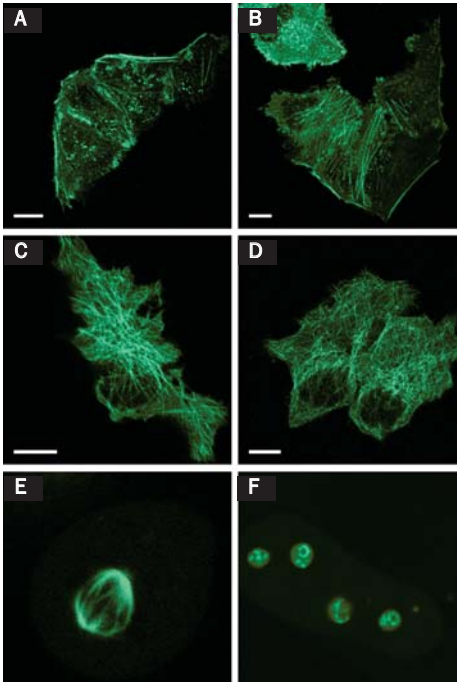


HEK293 Phoenix Eco cells were transiently transfected with Dendra2 gene under the control of CMV promoter. Dendra2 was converted to the red state in selected cells by brief illumination with 405-nm (left cell) or 488-nm (upper right and middle cells) lasers. Then confocal images of cells were made in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

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



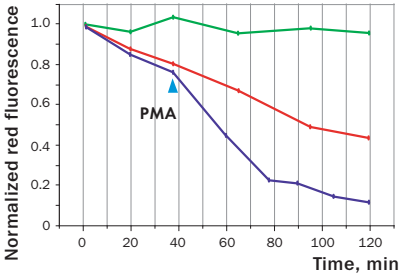
HeLa cells were transiently transfected with Dendra2 gene under the control of CMV promoter. Dendra2 was converted in a nucleus by brief illumination with 405-nm laser. Then confocal images of a cell were made in green and red channels and overlaid.



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.

Dendra2 use for determination of protein half-life

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 cycloheximid chase. It has been shown earlier that the phorbol ester, phorbol 12-myristate 13-acetate (PMA), increases the IkappaB-

alpha 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.

Dendra2 visualization, photoactivation and tracking in a confocal microscope

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING

Important note: This protocol has been optimized for Leica confocal inverted microscope DMIRE2 TCS SP2 equipped with HCX PL APO lbd.BL 63x 1.4NA oil objective and 125 mW Ar and 1 mW HeNe lasers. Analogous parameters should be used for other confocal microscopes.

A. Primary visualization of Dendra2 and Dendra2-tagged proteins

1. Bring cells into focus using white light illumination.

2. Switch off white light.

3. Select FITC or analogous filter set and open shatter of mercury lamp. Use strongly attenuated light (Intense blue light from the lamp may result in instant Dendra2 photoconversion).

4. Find cell(s) of interest expressing Dendra2 construct. Avoid prolonged observation of the cells.

5. Switch to confocal mode.

6. Use the following settings:

   Mode: xyt;

   Format: 512 x 512 or 1024 x 1024;

Zoom: 1;

Scan speed: 400 Hz;

Beam expander: 3;

Pinhole: 50-150 mm;

PMT1: 500-550 nm; gain 750-850 V (for green fluorescence detection);

PMT2: 560-670 nm; gain 750-850 V (for red fluorescence detection);

Averaging: 1 or 2.

7. Obtain images in green and red channels separately. For green channel use laser line 488 nm of 2-7% power (the less the better, depending on green signal brightness) and PMT1. For red channel use laser line 543 nm of 20-100% power and PMT2. To avoid cross-talk always set 0% power for 488 nm laser when obtain red image. Use sequential mode to obtain green and red images simultaneously.

Note: Ideally, you should obtain a clear cell image in green channel and no signal above background in red channel. If you see the same pattern for green and red fluorescence in the cell(s) of interest, thus Dendra2 photo-conversion already occurred during primary visualization (step A.4). In this case you should change the field of view using continuous scanning with 488 nm laser to find non-irradiated Dendra2-expressing cells.

8. Finally, select non-activated cell of interest and increase zoom to fit the cell. To reduce background in red channel a pre-irradiation with intense 543 nm laser line or green light from mercury lamp (TRITC filter set or similar) can be used. Obtain "before photoconversion" images in both green and red channels. Use sequential mode to obtain green and red images simultaneously.

B. Photoactivation of Dendra2 and Dendra2-tagged proteins

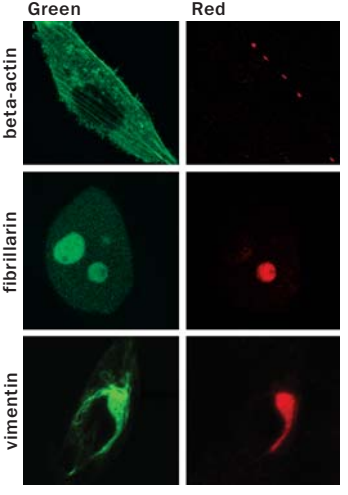
Dendra2 can be photoconverted by light in either UV-violet (360-420 nm) or blue region (460-500 nm). We recommend using

405 nm diode laser or 488 nm Ar laser line. 405-nm laser provides for much more efficient photoconversion compared to 488-nm laser. However, 405-nm laser represents still quite rare equipment while virtually all confocal microscopes have 488-nm laser. The protocols below are developed for activation at 488 nm (but it can be readily adapted for 405 nm laser).

Dendra2 local photoactivation can be done in two different ways described in two subsections below. We recommend using "point" activation (B.1) as it ensures much faster photoconversion which is extremely important for fast-moving proteins.

B.1. Activation at a selected point by high-intensity 488 nm light

Use a special mode when laser beam parks at a single selected pixel for a definite time (from 1 ms to min; note the strong



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.

difference compared to scanning mode with microsecond dwell time per pixel).

1. Set 488 nm laser line power for 20-50%.

2. Open "Bleach" dialog window and select point(s) of irradiation (it may be within the brightest area of the cell primary green image) and duration of irradiation (typically 50-200 ms). Press "Define" button. Close the window.

3. Restore settings for obtaining red image or both green and red images in sequential mode (step A.8).

4. Open "Advanced Time-Lapse" dialog window. In Lapse 1 click on the "Define" button and activate the "Bleach" check box. Press "Start" button.

Note: Ideally, you should detect bright red signal and partially bleached green signal in activation point(s). To select optimal activation conditions, different 488 nm laser power and different durations of irradiation should be tested. Too low illumination will result in low or zero conversion. Too high illumination will bleach the red signal - in this case you can see black point bordered with red signal.

B.2. Scanning a selected region with high-intensity 488 nm light

If point bleach mode with a parked laser beam is not available on your microscope, slow scan mode (long pixel dwelling time) should be applied.

Scan a selected (small) area within the cell to induce green-to-red conversion in this area.

1. Switch scan mode to the longest possible.

2. Zoom in a selected small region within the cell of interest. Use maximal or close to maximal zoom. Do not use ROI (region of interest) scanning mode without zooming.

3. Scan zoomed region with 20-50% power 488 nm laser for 1-7 times.

4. Undo zoom

5. Obtain images in green and red channels using the same setting as for "before photoconversion" images (step A.8).

Note: Ideally, you should detect bright red signal and partially bleached green signal in activation region. To select optimal activation conditions, different 488 nm laser power should be tested. Too low power will result in low or zero conversion. Too high power will bleach the red signal - in this case you can see black square bordered with red signal.

C. Visualization of Dendra2 and Dendra2-tagged proteins after photoactivation

After photoconversion you should immediately start time series of images that will provide information on migration of activated red Dendra2 from activation region and non-activated green Dendra2 into activation region. The same settings as for "before photoconversion" images (step A.8) should be used. Use sequential mode to monitor both green and red channels. Time interval between images should be selected depending on speed of target protein movement. Generally, 10-30 consecutive images are enough to measure protein mobility and at the same time avoid considerable photobleaching.

References

Chudakov *et al*. (2007) Nat. Protocols 8: 2024-2032.

Gurskaya *et al*. (2006) Nat. Biotechnol. 24(4):461-465.

Zhang *et al*. (2007) BioTechniques 42:446-450.