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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>26 kDa</td>
</tr>
<tr>
<td>Polypeptide length</td>
<td>230 aa</td>
</tr>
<tr>
<td>Structure</td>
<td>Monomer</td>
</tr>
<tr>
<td>Aggregation</td>
<td>No</td>
</tr>
<tr>
<td>Maturation rate at 37°C</td>
<td>Fast</td>
</tr>
<tr>
<td>Activating light</td>
<td>UV-violet (e.g. 405 nm) or blue (e.g. 488 nm)</td>
</tr>
<tr>
<td>Contrast, fold</td>
<td>Up to 4000</td>
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</table>

Fluorescence color: before photoconversion - green, after photoconversion - red

Excitation max: 490 nm
Emission max: 507 nm
Quantum yield: 0.50
Extinction coefficient, M^-1 cm^-1: 45 000
Brightness*: 22.5
pKa: 6.6

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

**References**
## Dendra2-related products

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat.#</th>
<th>Description</th>
<th>Size</th>
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</thead>
<tbody>
<tr>
<td><strong>Dendra2 expression/source vectors</strong></td>
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<tr>
<td>pDendra2-C*</td>
<td>FP821</td>
<td>Mammalian expression vector encoding humanized Dendra2 and allowing Dendra2 expression and generation of fusions to the Dendra2 C-terminus</td>
<td>20 μg</td>
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<tr>
<td>pDendra2-N*</td>
<td>FP822</td>
<td>Mammalian expression vector encoding humanized Dendra2 and allowing Dendra2 expression and generation of fusions to the Dendra2 N-terminus</td>
<td>20 μg</td>
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<tr>
<td>pDendra2-B</td>
<td>FP823</td>
<td>Bacterial expression vector; source of the humanized Dendra2 coding sequence</td>
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<tr>
<td>Gateway® Dendra2-At-C</td>
<td>FP824</td>
<td>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</td>
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<tr>
<td>Gateway® Dendra2-At-N</td>
<td>FP825</td>
<td>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</td>
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<td><strong>Recombinant protein</strong></td>
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<tr>
<td>rDendra2</td>
<td>FP852</td>
<td>Purified recombinant green-to-red photoswitchable protein</td>
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<td><strong>Antibodies against Dendra2</strong></td>
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<tr>
<td>Anti-Dendra2 antibody</td>
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<td>Rabbit polyclonal antibody against Dendra2</td>
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<td>AB822</td>
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<td>200 μg</td>
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Please contact your local distributor for exact prices and delivery information.

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### Notice to Purchaser:

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