

Dendra2 Protocol-at-a-Glance (PT5019-2)

This protocol is provided for use with the pDendra2 Vectors (Cat. Nos. 632544, 632545 & 632546) which encode a green-to-red photoswitchable fluorescent protein derived from the octocoral *Dendronephthya* sp. Dendra2 switches irreversibly from green to red when exposed to intense 405 or 488 nm light.

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

A. Experimental Setup

Transfect the Dendra2 construct into your cells using your method of choice. Mammalian cells transiently transfected with Dendra2 display an evenly distributed green signal within 10–12 hr posttransfection. Dendra2 matures efficiently at 20°C or 37°C, making it applicable to a wide range of experimental systems.

B. Visualizing Green (Unswitched) Dendra2

1. Bring cells into focus using white light illumination, then switch off the white light.
2. Select the FITC (or analogous) green filter set and open the shutter of the excitation light source. Use strongly attenuated blue light. Intense blue light from the lamp may result in instant Dendra2 photoconversion and is not recommended.
3. Find cell(s) of interest expressing green Dendra2 by monitoring for green fluorescence. Avoid prolonged visualization of the cells.
4. Switch to confocal mode, using settings appropriate to your microscope.

NOTE: The following settings are optimized for the Leica confocal inverted microscope DMIRE2 TCS SP2. Analogous parameters should be used for other microscopes.

- Mode: xyt
 - Format: 512 x 512 or 1024 x 1024
 - Zoom: 1
 - Scan speed: 400 Hz
 - Beam expander: 3
 - Pinhole: 50150 mm
 - PMT1: 500550 nm; gain 750850 V (for green fluorescence detection)
 - PMT2: 560670 nm; gain 750850 V (for red fluorescence detection)
 - Averaging: 1 or 2
5. Obtain images of the same field in the green and red channels.
 - For the green channel use a 488 nm laser line at 2–7% power and PMT1. Use the lowest possible intensity that still allows you to obtain a green fluorescent signal.
 - For the red channel, use a 543 nm laser line at 20–100% power and PMT2. To avoid crosstalk from green fluorescence, set the 488 nm laser to 0% power while you obtain the red image.

Ideally, you should obtain a clear image of the cell in the green channel, and no signal above background in the red channel. If you see the same pattern of green and red fluorescence in the cell(s) of interest, Dendra2 photoconversion has already occurred. In this case, change the field of view using continuous scanning with the 488 nm laser to find green (non-photoconverted) Dendra2-expressing cells.



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C. Switching Dendra2 From Green to Red

Identify an object showing green fluorescence only, and convert Dendra2 to its red form by exposure to either UV light (360–420 nm) or blue light (460–500 nm) using high-power light.

Switching can be done in two ways, as described below. We recommend using "point" activation (Protocol C.1, below) because it ensures much faster conversion to the red form, which is important for monitoring fast protein translocation events.

1. Point activation using high-intensity 488 nm light:

This protocol requires you to use a mode where the laser beam pauses at a single selected pixel for a defined length of time (typically 50–200 milliseconds). By comparison, scanning modes only remain on a particular pixel for microsecond(s).

- a. Set the 488 nm laser line at 20–50% power.
- b. Open the "Bleach" dialog window and select the point(s) you wish to switch to the red form of Dendra2 (typically, within the brightest green area) and the length of time for irradiation (typically 50–200 ms). Close the window.
- c. Restore the visualization settings used in Step B.5 for obtaining the red image only, or both green and red images in sequential mode.
- d. Open the "AdvancedTime_Lapse" dialog window. In Lapse 1, click on the "Define" button and activate the "Bleach" checkbox. Press "Start".

Ideally, you should detect a bright red signal and a partially bleached green signal at the activation point(s). To select the optimal activation conditions, test different % laser power and durations of irradiation. Too-weak illumination will result in low or zero conversion. Too-strong illumination will bleach the red signal, in which case you will see a black point bordered with red.

2. Scanning activation using high-intensity 488 nm light:

If your microscope cannot pause the laser beam at a specified pixel, apply a slow scan mode (long pixel dwelling time). Scan a selected (small) area within the cell to induce green-to-red photoconversion in this area.

- a. Choose the longest scan time available.
- b. Zoom in on a small region of interest (ROI) within the cell. Use maximal or close-to-maximal zoom. Do not use the ROI scanning mode without zooming.
- c. Scan the zoomed-in region up to 7 times with the 488 nm laser, using 20–50% power. To select optimal activation conditions, test different 488 nm laser power levels.
- d. Undo the zoom.

Ideally, you should detect a bright red signal and a partially bleached green signal at the activation point(s). To select the optimal activation conditions, test different % laser power and numbers of scans. Too-weak illumination will result in low or zero photoconversion. Too-strong illumination will bleach the red signal, in which case you will see a black point bordered with red.

D. Visualizing Red (Switched) Dendra2

Obtain images in the green and red channels using the same visualization settings used in Step B.5. After completing Protocol C.1 or C.2, restore the visualization settings used in Step B.5. Then monitor the movement of green and red Dendra2.

- Use the 488 nm laser at low power to excite and monitor green (e.g., unswitched) Dendra2.
- Use the 543 nm laser at higher power (20–100%) to excite and monitor red (e.g., switched) Dendra2.
- If you are performing a time series, decide how often to capture images based on the target protein's speed. Generally, 10–30 consecutive images are enough to measure protein mobility without causing significant photobleaching.

Notes About Filter Sets

- The green form of Dendra2 has excitation and emission maxima at 490 nm and 507 nm respectively, similar to other green fluorescent proteins. Thus, FITC and other commonly used filter sets for green dyes are ideally suitable for visualizing green Dendra2.
- The red form of Dendra2 has excitation and emission maxima at 553 nm and 573 nm respectively. Thus, a TRITC filter set or similar can be used to visualize red Dendra2.

Notes About Lasers

- Because 488 nm lasers are more common than diode lasers, we have given instructions for converting Dendra2 using 488 nm irradiation. A 405 nm laser provides more efficient photoconversion; however, a 488 nm laser is sufficient. If you prefer, this protocol is easily adapted for use with a 405 nm laser.
- The intense UV-violet light produced by a 405 nm laser can be harmful to cells.

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