Recombinant cyan-to-green photoswitchable fluorescent protein rDendra2

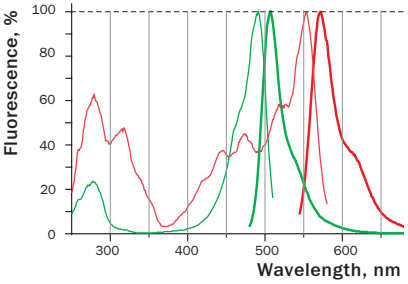
Cat.# FP851

- **Standard on protein gels and Western blots**
- **Control for fluorescence microscopy**
- **Calibration of fluorometers and FACS machines**
- **Microinjection into cells and tissues**

Recombinant Dendra2 (rDendra2) is 26-kDa green-to-red photoswitchable fluorescent protein. It has spectral properties identical to those of the expressed Dendra2 and is suitable as control reagent for Dendra2 expression using the Dendra2 expression vectors.

rDendra2 is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography. This method ensures high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption.

Storage: at +4°C in the dark place (before photoactivation).



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.

rDendra2 as standard on protein gel

As a standard on protein gel, the recombinant protein can be used to correlate Dendra2 expression levels to fluorescence intensity or to differentiate problems with detection of Dendra2 fluorescence from expression of Dendra2 protein.

When denatured by heating (2-3 min, 95-98°C), rDendra2 is detected as a single 27 kDa band on Coomassie stained SDS gel. If rDendra2 is to be used as an internal standard in a Coomassie-stained minigel, we recommend loading 0.5-1.0 µg of rDendra2 per lane. If rDendra2 is added to a total cell/tissue lysate or other crude sample, the amount of total protein loaded per lane must be optimized for the particular application.

Predenatured rDendra2 will not fluoresce on SDS gel, whereas nondenatured one will fluoresce on SDS gel.

rDendra2 as control for fluorescence microscopy

The purified proteins may be used to optimize lamp and filter set conditions for detection of Dendra2 fluorescence, or as a qualitative means to correlate Dendra2 fluorescence with protein amount in transfected cells.

A. Unfixed samples

Please use this method for live cell fluorescence or other cases where a fixation step is not desired

1. Perform 1:10 serial dilutions of the 1.0 mg/ml rDendra2 stock solution with 10 mM Tris-HCl (pH 8.0) to yield concentrations of 0.1 mg/ml and 0.01 mg/ml.

Notes:

- These dilutions should suffice as a positive control. The 1.0 mg/ml solution will give a very bright fluorescent signal by microscopy.
- The diluted samples can be stored at +4°C for up to 3 months with no loss of fluorescence intensity.

2. Using a micropipette, spot 1-2 µl of diluted protein onto the microscope slide. If slide contains a mounted coverslip, position the spot several millimeters away from the sample such that a second coverslip can be added over the protein spot.

3. Allow the protein to air-dry for a few seconds, and mark the position of the spot on the other side of the slide to aid in focusing.

4. Add a coverslip over the spot using a 90% glycerol solution in 100 mM Tris-HCl (pH 7.5).

5. Fluorescence from the spot is best viewed at low magnification, using either a 10X or 20X objective lens.

B. Fixed samples

In some cases it may be necessary to fix the recombinant protein to the microscope slide prior to microscopy. This can be done by dipping the section of the microscope slide containing the air-dried protein spot (after Step A3 above) into 100% methanol for 1 min. Allow the slide to dry completely and place a coverslip over the sample as in Step A4 above.

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