

# Recombinant yellow fluorescent protein rPhiYFP

Cat.# FP651

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

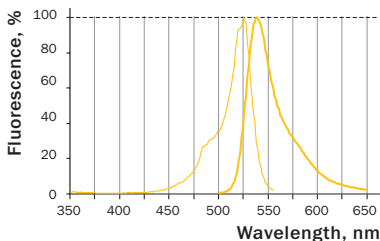
Recombinant PhiYFP (rPhiYFP) is 26-kDa yellow fluorescent protein. It has spectral properties identical to those of the expressed PhiYFP and is suitable as control reagent for PhiYFP expression using the PhiYFP expression vectors.

rPhiYFP is purified from transformed *E. coli* using organic extraction and hydrophobic chromatography or metal-ion affinity chromatography (methods vary for different lots). Both methods ensure high purity of the recombinant protein and maintenance of fluorescence. The protein concentration is measured by chromophore absorption. rPhiYFP may contain 6xHis tag at its N-terminus (vary in different lots).

**Storage:** at +4°C

**Normalized excitation (thin line) and emission (thick line) spectra of Phi-Yellow proteins.**

Complete PhiYFP and PhiYFP-m spectra in Excel format can be downloaded from the Evrogen Web site at [www.evrogen.com/PhiYFP.shtml](http://www.evrogen.com/PhiYFP.shtml)



### **rPhiYFP as standard on protein gel**

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

When denatured by heating (2-3 min, 95-98°C), rPhiYFP is detected as a single 26 kDa band on Coomassie stained SDS gel. If rPhiYFP is to be used as an internal standard in a Coomassie-stained minigel, we recommend loading 0.5-1.0 µg of rPhiYFP per lane. If rPhiYFP 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 rPhiYFP will not fluoresce on SDS gel, whereas nondenatured one will fluoresce on SDS gel.

### **rPhiYFP as control for fluorescence microscopy**

The following protocols are for rPhiYFP use as a control on microscope slides in fluorescence microscopy. The purified proteins may be used to optimize lamp and filter set conditions for detection of PhiYFP fluorescence, or as a qualitative means to correlate PhiYFP 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 rPhiYFP 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  $\mu$ l 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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