Recombinant green fluorescent protein rTagGFP2

Cat. # FP152

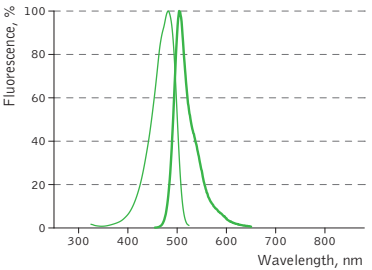
Amount:	**100 µg**
Concentration:	**1.0 mg/ml**
Storage buffer:	**1 x PBS**
Storage conditions:	**store at +4°C**
Shipping conditions:	**ambient temperature**
Shelf life:	**6 months from date of receipt under proper storage conditions**
Lot number:	**specified on product label**

Recombinant TagGFP2 (rTagGFP2) is 27-kDa green fluorescent protein. It has spectral properties identical to those of the expressed TagGFP2 and is suitable as control reagent for TagGFP2 expression using the TagGFP2 expression vectors.

rTagGFP2 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. rTagGFP2 contains 6xHis tag at its N-terminus.

TagGFP2 normalized excitation (thin line) and emission (thick line) spectra.

Complete TagGFP2 spectra in Excel format can be downloaded from the Evrogen Web site www.evrogen.com



rTagGFP2 as standard on protein gel

As a standard on protein gel, the recombinant protein can be used to correlate TagGFP2 expression levels to fluorescence intensity or to differentiate problems with detection of TagGFP2 fluorescence from expression of TagGFP2 protein.

When denatured by heating (2-3 min, 95-98°C), rTagGFP2 is detected as a single 27 kDa band on Coomassie stained SDS gel. If rTagGFP2 is to be used as an internal standard in a Coomassie-stained minigel, we recommend loading 0.5-1.0 µg of rTagGFP2 per lane. If rTagGFP2 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.

Unlike native protein, pre-denatured rTagGFP2 does not fluoresce on SDS gel.

rTagGFP2 as control for fluorescence microscopy

The following protocols are for rTagGFP2 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 TagGFP2 fluorescence, or as a qualitative means to correlate TagGFP2 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.

A.1. Perform 1:10 serial dilutions of the 1.0 mg/ml rTagGFP2 stock solution with 10 mM TrisHCl (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.

A.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.

A.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.

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

A.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 point A.3. above) into 100% methanol for 1 min. Allow the slide to dry completely and place a coverslip over the sample as in point A.4. above.

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