



Recombinant red fluorescent protein rTagRFP

Cat. # FP154

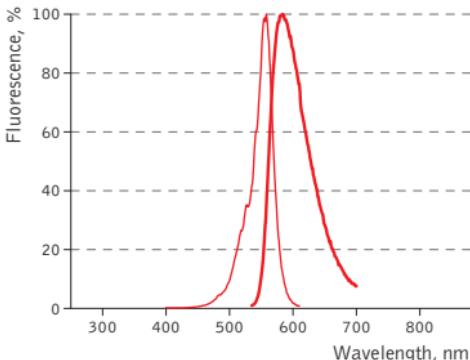
Amount:	100 µg
Concentration:	1.0 mg/ml
Storage buffer:	1 x PBS
Storage conditions:	store at +4°C
Shipping conditions:	ambient temperature
Shelf life:	6 months from date of receipt under proper storage conditions
Lot number:	specified on product label

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

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

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

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



rTagRFP on protein gel

When denatured by heating (2-3 min, 95-98°C), rTagRFP demonstrate partial fragmentation with a break point just before the chromophore. It leads to the presence of multiple bands on Coomassie stained SDS gel. These bands correspond to truncated and partially truncated forms of rTagRFP.

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

rTagRFP as control for fluorescence microscopy

The following protocols are for rTagRFP 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 TagRFP fluorescence, or as a qualitative means to correlate TagRFP 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 rTagRFP 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.

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 Tris-HCl (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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