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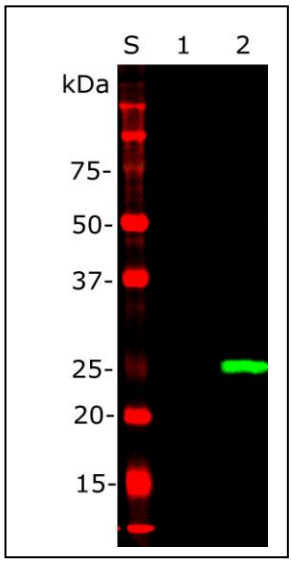
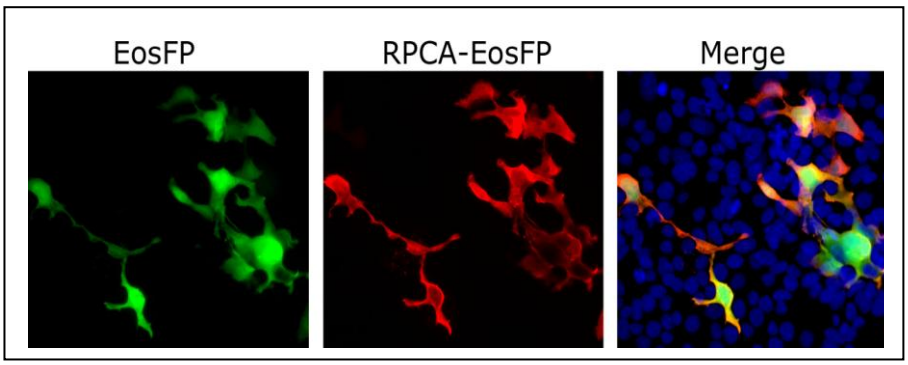
Catalogue# RPCA-EosEP: Rabbit Polyclonal Antibody to Green-Red photoconvertible fluorescent protein EosFP

Immunogen: Fluorescent proteins have become widely used in a variety of experimental paradigms since the characterization of the first one, Green Fluorescent Protein (GFP) about 20 years ago. Some of these fluorescent proteins have the useful property of changing their emission spectrum from green to red following irradiation with blue or UV light, which allows simple but powerful pulse chase type experiments to be performed using more or standard fluorescence microscopes. We have expressed in *E. coli* an purified a protein originally isolated from *Lobophyllia hemprichii*, a type of stony coral. The protein was described in Wiedenannn et al. (2004) and named EosFP, after Eos, the goddess of dawn in Greek mythology. Upon appropriate irradiation the protein changes emission from 516 nm to 581 nm, which happens to fit very conveniently to typical green (~498 nm) and red (~594 nm) filters on fluorescence microscopes. The emission shift is due to an irreversible covalent modification in the fluorochrome and is dependent on the His residue in the His-Tyr-Gly sequence that produces the fluorescence. The original coral protein was mutagenized to prevent tetramerization and dimerization, a requirement if the protein is to be used for fusion protein generation useful for FRET and similar techniques. A recent advance was the development of CaMPARI, an acronym for "Calcium-Modulated Photoactivatable Ratiometric Integrator" (2). This construct contains the GCaMP Calcium indicator fused to two EosFP domains, and will only transit from green to red when there is a coincidence between Calcium level elevation and the appropriate wavelength of light. This allows functional mapping of cellular activation in real time in appropriate transgenic animals (2).





Left: Western blot analysis of RPCA-EosFP. 1: Non-transfected HEK293. 2: Transfected HEK293 cells which overexpress protein EosFP. S: Protein standard of indicated molecular weight. RPCA-EosFP at 1: 1,000 dilution. There is a strong clean band at ~25 kDa corresponding to EosFP in transfected cells, but not in non-transfected cells. **Right**: Transfected HEK293 cells which overexpress protein EosFP were stained with RPCA-EosFP and viewed in the microscope. Cells which are transfected with EosFP (left panel) are bright green. On staining with RPCA-EosFP in red (middle panel), cells appear orange (right panel). Most HEK293 cells are not transfected so only the nucleus of these cells can be visualized with a blue DNA stain. The red antibody staining is only seen in cells which express EosFP, as expected, and the superimposition of the green and red signals results in the orange color.

Antibody Characteristics: Antibody was raised in rabbit, and is provided as crude serum. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: Try at dilutions of 1:1,000-1:5,000 for immunofluorescence. For western blots try at 1:1,000.

References:

1: Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. Science 263:802-5 (1994).

2: Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML and Lukyanov SA. Fluorescent proteins from nonbiolu.minescent Anthozoa species Nat. Biotechnol. 17: 969-973 (1999).

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.