

## Blue fluorescent protein TagBFP

- Bright blue fluorescence
- Monomeric fast maturing protein with successful performance in fusions
- Extremely high pH-stability
- High photostability
- Recommended as a donor for green fluorescent proteins in FRET applications

TagBFP (scientific name mTagBFP) is a monomeric blue fluorescent protein generated by site-specific and random mutagenesis of TagRFP [Subach et al. 2008]. TagBFP possesses bright blue fluorescence with excitation/emission maxima at 402 and 457 nm, characterized by high photostability and extremely high pH-stability.

Compared to EBFP2 [Ai et al. 2007], TagBFP is more than 1.8 times brighter, much more pH-stable and has twice shorter maturation half-time at 37°C. Narrow fluorescence emission peak of TagBFP provides for accurate and easy spectral separation with cyan and green fluorescent proteins and makes it a preferable tag for multicolor labeling.

Good overlap between the emission spectrum of TagBFP and the absorbance spectra of TagGFP allows using these two proteins as a FRET pair.

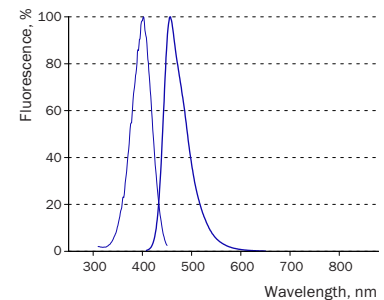
### Main properties of TagBFP

| Characteristic                          |         |
|---|---------|
| Molecular weight, kDa                   | 26      |
| Polypeptide length, aa                  | 233     |
| Fluorescence color                      | blue    |
| Excitation maximum, nm                  | 402     |
| Emission maximum, nm                    | 457     |
| Quantum yield                           | 0.63    |
| Extinction coefficient, $M^{-1}cm^{-1}$ | 52 000  |
| Brightness*                             | 32.8    |
| Brightness, % of EGFP                   | 99      |
| pKa                                     | 2.7     |
| Structure                               | monomer |
| Aggregation                             | no      |
| Maturation rate at 37°C                 | fast    |
| Photostability                          | high    |

\*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

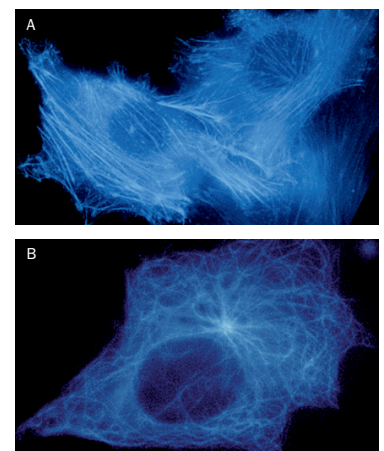
TagBFP can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with TagBFP expression vectors give bright fluorescent signals within 10-12 hrs after transfection. No cell toxic effects and visible protein aggregation are observed. TagBFP performance in fusions has been demonstrated in the  $\beta$ -actin and  $\alpha$ -tubulin models. It can be used in multicolor labeling applications with green, yellow, red, and far-red fluorescent dyes.

TagBFP can be successfully used as a donor in FRET pair also comprising TagGFP. The calculated Forster distance  $R_0 = 5.25$  nm for TagBFP / TagGFP pair is larger than those reported for the standard ECFP-EYFP and mCyPet-mYPet pairs ( $R_0 = 4.86$  nm and 4.93 nm correspondingly), suggesting that TagBFP / TagGFP is one of the best among available FRET pairs of monomeric fluorescent proteins. High efficiency of TagBFP as a FRET donor was demonstrated in living cells by Subach et al. [Subach et al. 2008].



**TagBFP normalized excitation (thin line) and emission (thick line) spectra.**

Complete TagBFP spectra in Excel format can be downloaded from the Evrogen Web site at [www.evrogen.com / support / FP-tech.shtml](http://www.evrogen.com/support/FP-tech.shtml)



**HeLa cells expressing TagBFP fusion proteins.**

(A) - confocal microscopy of TagBFP fusion with  $\beta$ -actin in transiently transfected HeLa cells; (B) - confocal microscopy of TagBFP fusion with cytoplasmic  $\alpha$ -tubulin in transiently transfected HeLa cells.

### Recommended filter sets and antibodies

The protein can be recognized using Anti-tRFP antibody (Cat.# AB233-AB234) available from Evrogen.

TagBFP can be detected using common fluorescence filter sets for BFP, DAPI, and other blue dyes.

**Recommended filter sets are:** XF119-2\*, QMAX-Blue\*, XF131, XF06, XF13-2, XF03, XF11, XF129-2, XF05-2 (Omega Optical); DAPI-5060B\* and DAPI-1160A (Semrock); 31037, 31041, 31016\*, 31021, 31000v2, 1009v2, 31013v2, 11005v2, 31047 (Chroma Technology Corp.).

\* - preferred filter sets

### Available variants and fusions

TagBFP codon usage is optimized for high expression in mammalian cells [Haas et al. 1996], but it can be successfully expressed in many other heterologous systems.

**TagBFP-actin fusion:** Human  $\beta$ -actin is linked to the TagBFP C-terminus. When expressed in mammalian cells, this fusion provides blue fluorescent labeling of actin filaments.

**TagBFP-tubulin fusion:** Human  $\alpha$ -tubulin is linked to the TagBFP C-terminus. When expressed in mammalian cells, this fusion provides blue fluorescent labeling of tubulin filaments.

### TagBFP-related products

| Product                                 | Cat.# | Description  | Size        |
|---|-------|--|-------------|
| <i>TagBFP expression/source vectors</i> |       |  |             |
| pTagBFP-C                               | FP171 | Mammalian expression vector encoding humanized TagBFP and allowing its expression and generation of fusions to the TagBFP C-terminus | 20 $\mu$ g  |
| pTagBFP-N                               | FP172 | Mammalian expression vector encoding humanized TagBFP and allowing its expression and generation of fusions to the TagBFP N-terminus | 20 $\mu$ g  |
| pTagBFP-actin                           | FP174 | Mammalian expression vector encoding humanized TagBFP fused with human cytoplasmic $\beta$ -actin                                    | 20 $\mu$ g  |
| pTagBFP-tubulin                         | FP175 | Mammalian expression vector encoding humanized TagBFP fused with human $\alpha$ -tubulin   | 20 $\mu$ g  |
| <i>Antibodies against TagBFP</i>        |       |  |             |
| Anti-tRFP                               | AB233 | Rabbit polyclonal antibody against TurboRFP, TurboFP602, TurboFP635, TagBFP, TagRFP,   | 100 $\mu$ g |
|   | AB234 | and TagFP635   | 200 $\mu$ g |

The prices do not include delivery. The prices vary in different countries. Please contact your local distributor for exact prices and delivery information.

### References

Ai et al. (2007) "Exploration of new chromophore structures leads to the identification of improved blue fluorescent proteins." *Biochemistry*, 46 (20): 5904–10 / pmid: 17444659

Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315–24 / pmid: 8805248

Subach et al. (2008) "Conversion of Red Fluorescent Protein into a Bright Blue Probe." *Chemistry & Biology*, 15 (10): 1116–1124

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