

## BI

(Z)-3-((1H-Benzo[d]imidazol-4-yl)methyl)-5-(3,5-Difluoro-4-Hydroxybenzylidene)-2-Methyl-3,5-Dihydro-4H-Imidazol-4-One

Cat. No. 600-1mg



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## Product

BI is a DFHBI derivative that binds Broccoli™ with higher affinity and deliver improved cellular fluorescence. In cells, BI has been found to stabilize the Broccoli™ structure and promote its folding. Additionally, Broccoli™/BI complex is significantly more photostable due to impaired light-induced photoisomerization, and rapid unbinding of photoisomerized *cis*-BI. BI is cell-permeable with negligible toxicity in living cells and can be used to label any genetically encoded Broccoli™ RNA tag without disrupting biological functions. Importantly, the optimized fluorescence properties of BI enable live single-molecule imaging of Broccoli™-tagged mRNA transcripts in mammalian cells. Compared to MS2-tagged mRNA, Broccoli™-tagged mRNA puncta exhibit similar size and puncta diameters fall within a single Gaussian curve.

## Presentation

Each vial contains lyophilized BI dyes. Resuspension in DMSO at 50 mM concentration is recommended before transferring to the desired experimental buffer.

## Storage

Store at -20 °C. Stable for 2 years at -20 °C from the date of shipment. Non-hazardous. No MSDS needed.

## Specifications

Excitation maximum: 470 nm  
Emission maximum: 505 nm  
Extinction coefficient ( $M^{-1} cm^{-1}$ )<sup>a</sup> : 42,500  
Quantum yield: 0.67  
 $K_D$  : 51 nM  
Brightness<sup>b</sup> : 188

<sup>a</sup> Extinction coefficient of BI was measured in buffer containing 40 mM HEPES [7.4], 100 mM KCl, 1 mM MgCl<sub>2</sub>.

<sup>b</sup> Brightness is relative to Broccoli™/DFHBI-1T.

## Data

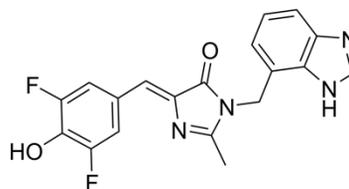


Figure 1. Structure of BI. MW = 368.3

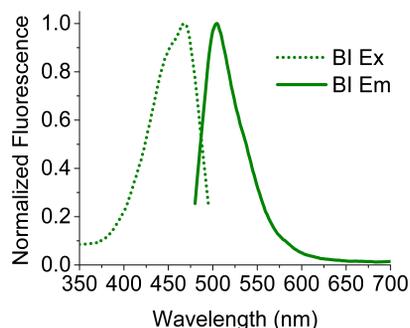


Figure 2. Excitation and emission spectra of Broccoli™/BI complex.

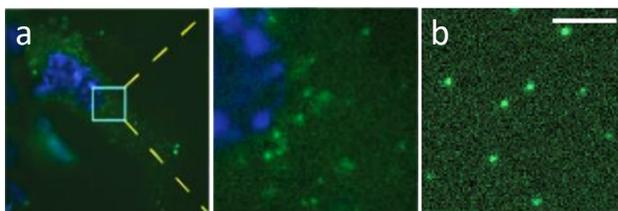


Figure 3. a) Live COS7 cells expressing  $\beta$ -actin-Broc<sub>24x</sub> transcripts were imaged in the presence of 10  $\mu$ M BI.  $\beta$ -actin mRNA can be detected as mobile Broccoli puncta (green) that localized mostly in the cytosol. Cell nucleus is labeled with Hoechst stain (blue). b) Broccoli-tagged mRNA puncta reflects single mRNA molecule distribution and sizing.

## References

Li X, et al. 2020. Fluorophore-promoted RNA folding and photostability enable imaging of single Broccoli-tagged mRNAs in living mammalian cells. *Angew Chem Int Ed* 59(11), 4511-4518.