



## Next Generation Fluorescence Imaging

Smart Sensor Solutions

### General Information

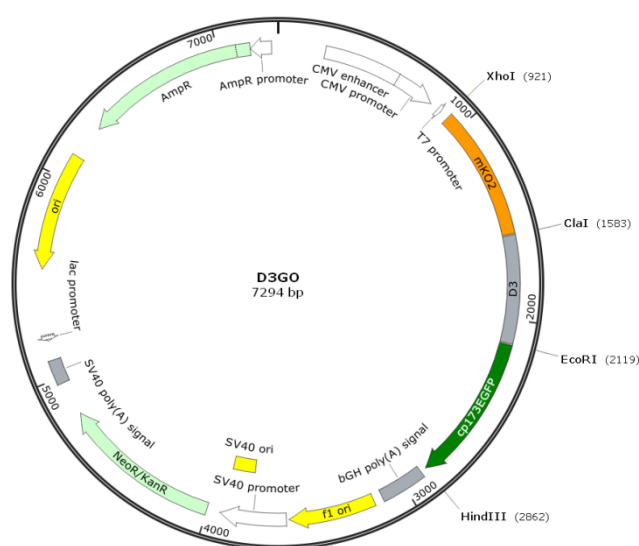
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial use.

### D3GO-Cam vector

This vector has not been completely sequenced. All provided information regarding the vector composition was compiled using the information from published literature, other sources together with partial sequences obtained by NGFI.

### Vector description

D3GO-Cam is a genetically encoded FRET-based probe for imaging cytosolic  $\text{Ca}^{2+}$  with increased  $\text{Ca}^{2+}$  sensitivity of intact mammalian cells. In order to express D3GO-Cam in cells of interest, 20  $\mu\text{g}$  of purified endotoxin-free plasmid DNA coding for D3GO-Cam is provided. The plasmid coding for D3GO-Cam represents a mammalian expression vector with a strong viral promoter. For plasmid amplification in *E. coli* ampicillin should be used. 1 – 1.5  $\mu\text{g}$  DNA is required for cell transfection in a single well of a standard 6-well dish following standard transfection procedures. Usually cells express high amounts of D3GO-Cam 24 – 48 hours after cell transfection. Standard optical filters for GFP/OFOP or alternatively GFP/RFP FRET imaging should be used. The vector can be also used as a source of D3GO-Cam coding sequence. Flanking restriction sites are convenient for excision of D3GO-Cam sequence and its further insertion into other expression vectors of choice.



### Expression in mammalian cells

D3GO-Cam vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive D3GO-Cam expression in eukaryotic cells.

### Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to ampicillin (100  $\mu\text{g}/\text{ml}$ ) to *E. coli* hosts.

Note: The higher excitation wavelength of red-shifted cameleons ( $\sim 480 \text{ nm}$ ) allows a combination with UV-excitable chemical indicators such as fura-2 for simultaneous real-time imaging of  $\text{Ca}^{2+}$  dynamic within the cytosol in a ratiometric manner.

### References

Waldeck-Weiermair M. (2012) "Spatiotemporal Correlations between Cytosolic and Mitochondrial  $\text{Ca}^{2+}$  Signals Using a Novel Red-Shifted Mitochondrial Targeted Cameleon"  
(<http://journals.plos.org/plosone/article/asset?id=10.1371%2Fjournal.pone.0045917.PDF>)

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