



## Next Generation Fluorescence Imaging

Smart Sensor Solutions

### General Information

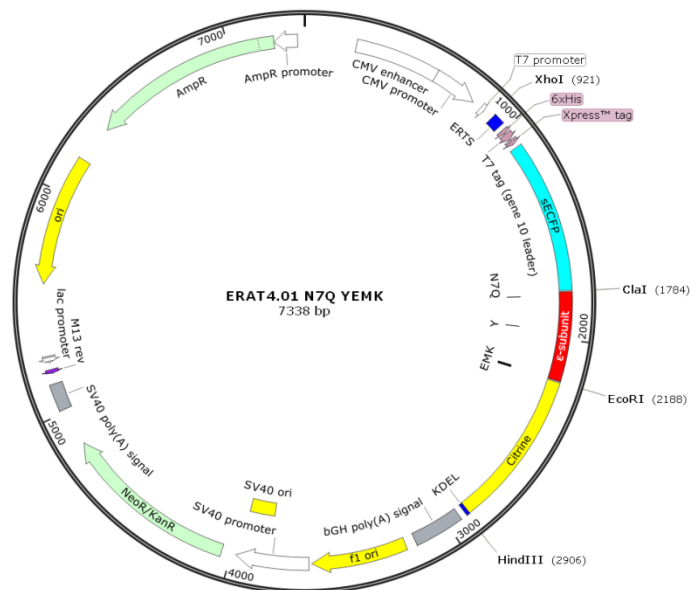
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial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

### ERAT4.01<sup>N7Q YEMK</sup> 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

ERAT4.01<sup>N7Q YEMK</sup> is a genetically encoded FRET-based probe for imaging ATP with increased sensitivity ( $K_d = 1.2\text{mM}$ ) within the endoplasmic reticulum (ER) of intact mammalian cells. In order to express ERAT4.01<sup>N7Q YEMK</sup> in cells of interest, 20  $\mu\text{g}$  of purified endotoxin-free plasmid DNA coding for ERAT4.01<sup>N7Q YEMK</sup> is provided. The plasmid coding for ERAT4.01<sup>N7Q YEMK</sup> 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 ERAT4.01<sup>N7Q</sup> 24 – 48 hours after cell transfection. Standard optical filters for CFP/YFP-FRET imaging should be used. The vector can be also used as a source of ERAT4.01<sup>N7Q YEMK</sup> coding sequence. Flanking restriction sites are convenient for excision of ERAT4.01<sup>N7Q YEMK</sup> sequence and its further insertion into other expression vectors of choice.



### Expression in mammalian cells

ERAT4.01<sup>N7Q YEMK</sup> vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive ERAT4.01<sup>N7Q YEMK</sup> 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: ERAT4.01<sup>N7Q YEMK</sup> vector contains a His-tag (6xHIS) between the ER targeting sequence (ERTS) and the ATP sensor to enable pull-down assays.

### References

Vishnu N. (2014) "ATP increases within the lumen of the endoplasmic reticulum upon intracellular  $\text{Ca}^{2+}$  release" (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3907277/pdf/368.pdf>)

Imamura H. (2009) "Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735558/pdf/zpq15651.pdf>)

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