



## Next Generation Fluorescence Imaging

Smart Sensor Solutions

### General Information

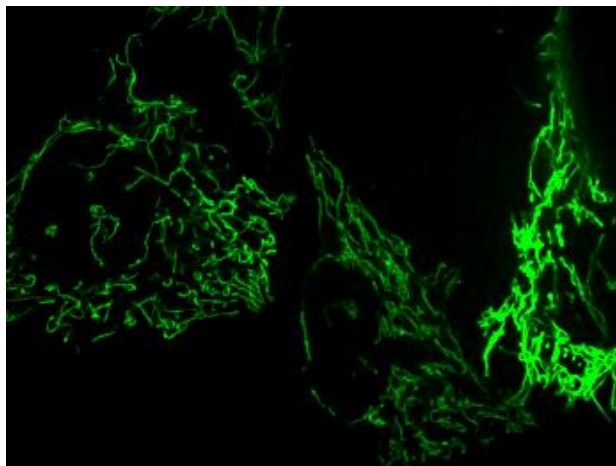
This recombinant human adenovirus type 5 expresses mtD1GO-Cam (mtD1GO-Cam AV5) under the control of a CMV promoter.

### Contents and Storage

Recombinant adenovirus is supplied in liquid form at indicated titer. Store at -80°C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer immediately. DO NOT FREEZE AND THAW REPEATEDLY.

### Description

mtD1GO-Cam is a red-shifted FRET based genetically encoded biosensor capable for specific detection of  $\text{Ca}^{2+}$  within the mitochondrial matrix of intact living mammalian cells. Infection of recombinant adenovirus encoding mtD1GO-Cam represents as an alternative for difficult plasmid transfection into mammalian cells with almost 100 % efficiency of gene delivery in most cell types. mtD1GO-Cam AV5 can be added directly to cells in culture medium in the presence or absence of serum. Usually cells express high amounts of mtD1GO-Cam 24 – 48 hours after cell infection. Standard optical filters for GFP/RFP FRET imaging should be used. mtD1GO-Cam AV5 can be used for subcloning mtD1GO-Cam.



*mtD1GO-Cam expression in adenovirus infected EA.hy926 cells, a somatic hybrid of Human Umbilical Vein Endothelial Cells (HUVEC).*

### Guideline for the infection of mtD1GO-Cam AV5

The amount of adenovirus cell receptors largely varies among different cell types and an optimal concentration needs to be determined for the cell line of choice. An appropriate amount of virus of infected cells is important for the outcome of experiments. The goal is to achieve a 100 % infection efficiency without causing cytotoxicity. Due to the emitting fluorescence of mtD1GO-Cam expressing cells this adenovirus is suitable for pilot testing the appropriate amount of virus for an individual mammalian cell type. We recommend to use different virus concentrations ranging from 1 to 1000 MOI (multiplicity of infection) and to determine the optimal amount by monitoring the orange fluorescence 24, 48 and 72 hours after infection. Most cell types like HEK293, HeLa or smooth muscle cells range between 1 and 100 MOI, but up to 1000 MOI may be used for lymphoid or endothelial cell lines.

### References

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

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Waldeck-Weiermair M. (2013) "Molecularly Distinct Routes of Mitochondrial  $\text{Ca}^{2+}$  Uptake Are Activated Depending on the Activity of the Sarco/Endoplasmic Reticulum  $\text{Ca}^{2+}$  ATPase (SERCA)"  
(<http://www.jbc.org/content/288/21/15367.full>)

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