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### SZABO-SCANDIC HandelsgmbH

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## FG 488 DHPE

®

MedChemExpress

Cat. No.:	HY-D1560	
CAS No.:	438476-80-3	~
Molecular Formula:	$C_{58}H_{82}F_{2}NO_{14}P$	
Molecular Weight:	1086.24	
Target:	Fluorescent Dye	PR P
Pathway:	Others	F T F
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.	но <sup>, , , , , , , , , , , , , , , , , , , </sup>

BIOLOGICAL ACTIVITY		
Description	FG 488 DHPE is a lipid-coupled fluorochrome, has be used as a fluorophore Oregon Green 488. FG 488 DHPE monitors acidification of lipid vesicles with λex/λem=508/534 nm.FG 488 DHPE is also used for Hv1-induced proton translocation quantificatio with λex/λem=508/534 nm as well <sup>[1][2]</sup> .	
In Vitro	<ul> <li>FG 488 DHPE shows a pH-dependent fluorescence emission characteristic<sup>[1]</sup>.</li> <li>Monitoring acidification in Bulk vesicle assa<sup>[1]</sup>: <ol> <li>I.Instrument: Jasco FP6500 spectrofluorometer, 37 BØ fluorescence is excited at λex=508 nm and the emission is detected at λem=534 nm.</li> <li>2.Add 100 µL proteoliposomes (cphospholipid is about 60 µM) to 680 µL ATPase buffer, containing the K+-ionophore valinomycin (5 nM) to enable a charge equilibration for transported protons.</li> <li>3.Add ATP (1.2 mM) to induce proton pumping.</li> <li>4.Add 1 mM NaN3 to ATP hydrolysis.</li> <li>5.Add CCCP (carbonyl cyanide 3-chlorophenyl hydrazine, 0.4 µM) to deplete the proton gradient.</li> <li>6.Conversion into pH-values, fluorescence intensities are normalized to the intensity obtained directly after ATP addition.</li> <li>FG 488 DHPE exerts function in quantification of pH changes induced by the voltage-dependent proton channel Hv1<sup>[2]</sup>.</li> <li>Quantification of phospholipid concentrations<sup>[2]</sup>:</li> <li>1.Add Perchloric acid (70%, 200 µL) to a sample of unilamellar vesicles containing OG488-DHPE (30 µL).</li> <li>2.Heat up to 220 °C for 60 min to generate inorganic phosphate.</li> <li>3.Cooling down to room temperature, add 700 µL of a solution of NH<sub>4</sub>MoO<sub>4</sub> (0.45% (w/v)) and perchloric acid (12.6% (w/v)) and 700 µL of a 1.7% (w/v) acetic acid solution.</li> <li>4.Obtain a calibration curve to know NAH<sub>2</sub>PO<sub>4</sub> concentrations.</li> <li>5.Incubated samples at 80 °C for 10 min and measure the absorption of the samples at 820 nm.</li> <li>6.Calculate phospholipid concentrations of the vesicles using the calibration curve.</li> </ol> </li> <li>Proton translocation assay<sup>[2]</sup>: <ol> <li>1.Instrument: Jasco FP6500 spectrofluorometer, 37 BØ fluorescence is excited at λex=508 nm (3 nm band width) and the emission is detected at λem=534 nm (3 nm band width).</li> <li>2.Dilute proteoliposomes composed of POPC/POPG/Chol/OG488-DHPE (54.5:25:20:0.5) in buffer A in flux buffer generating a 14-fold K+-gradient across the ve</li></ol></li></ul>	

channels as described above.
4.Add CCCP (6 nM) to permeabilise all vesicles for protons.
5. The normalized fluorescence intensity Fnorm is plotted as a function of time. As a control for proton leakage, protein-free
vesicles were used instead of proteoliposomes.
For the experiments in the presence of the potential inhibitor 2GBI, dissolve the inhibitor (15 mM) in flux buffer and add (0.5-
8.0 μL) to the proteoliposomes before addition of valinomycin to induce proton translocation.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### REFERENCES

[1]. Schwamborn M, et al. Monitoring ATPase induced pH changes in single proteoliposomes with the lipid-coupled fluorophore Oregon Green 488. Analyst. 2017 Jul 10;142(14):2670-2677.

[2]. Gerdes B, et al. Quantification of Hv1-induced proton translocation by a lipid-coupled Oregon Green 488-based assay. Anal Bioanal Chem. 2018 Oct;410(25):6497-6505.

Caution: Product has not been fully validated for medical applications. For research use only.

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