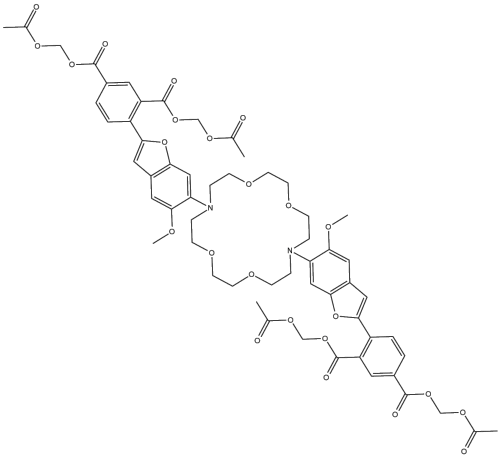
Product Data Sheet

Product Name: PBFI AM
Cat. No.: GC18477

Chemical Properties

Cas. No.	124549-23-1		
Chemical Name	4,4'-[1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diylbis(5-methoxy-6,2-benzofurandiyl)]bis-1,3-benzenedicarboxylic acid, tetrakis[(acetyloxy)methyl] ester		
SMILES	COC1=CC2=C(OC(C3=C(C(OCOC(C)=O)=O)C=C(C(OCOC(C)=O)=O)C=C3)=C2)C=C1N4CCOCCOCCN(C5=CC(OC(C6=C(C(OCOC(C)=O)=O)C=C(C(OCOC(C)=O)=O)C=C6)=C7)=C7C=C5OC)CCOCCOCC4		
Formula	$C_{58}H_{62}N_2O_{24}$	M.Wt	1171.1
Solubility	Soluble in methanol and DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

Intracellular K^+ determination applying the PBFI AM

This protocol only provides a guideline, and should be modified according to your specific needs.

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

(1) Take an appropriate amount of PBFI AM solid and dissolve the solid in DMSO to prepare a high-concentration stock solution (such as 1mM).

(2) 1mM PBFI AM stock solution is diluted into 10μM PBFI AM working solution using PBS buffer. The PBFI AM working solution should be prepared fresh for immediate use.

2. Cell sample preparation

1.5×10^4 cells are inoculated into 96-well culture plates and cultured overnight in complete medium (containing 10% fetal calf serum).

3. PBFI AM staining

(1) Replace the completed medium with low-serum (1%) medium and incubate for 45 minutes (37°C, 5% CO_2).

(2) Remove the culture medium and wash the cells 2-3 times with PBS.

(3) The cells are then loaded with PBFI AM by adding 50μl of 10μM PBFI AM in each well.

(4) Gently shake the petri dish to ensure the staining solution is evenly distributed.

(5) After 100 minutes, the PBFI AM working solution is aspirated and discarded, and the cells are washed 2-3 times with PBS.

(6) Add an appropriate amount of PBS buffer to immerse the cells.

4. Fluorescence detection

Excitation ratios of fluorescence at 344 and 400nm, measured at emission 500nm are used to estimate the intracellular content of K^+.

5. Notes

(1) PBFI AM is photosensitive. During the operation, avoid light as much as possible.

(2) The staining time and temperature should be optimized according to the cell type and

experimental requirements.

(3) During the experiment, the experimental conditions such as temperature, should be strictly controlled to ensure the reliability of the experimental results.

References:
[1] Andersson B, Janson V, Behnam-Motlagh P, et al. Induction of apoptosis by intracellular potassium ion depletion: using the fluorescent dye PBFI in a 96-well plate method in cultured lung cancer cells[J]. Toxicology in vitro, 2006, 20(6): 986-994.

Background

PBFI AM, the cell membrane permeable fluorescent probe, acts as the K(+)-sensitive dye [1]. The interaction of buforin IIb with Jurkat cell membranes was studied by measuring intracellular K^+ levels using PBFI AM [2]. PBFI AM can be used in flow cytometry analysis to investigate the correlation between cell apoptosis and potassium ion levels[3].

References:
[1] Kasner S E, Ganz M B. Regulation of intracellular potassium in mesangial cells: a fluorescence analysis using the dye, PBFI[J]. American Journal of Physiology-Renal Physiology, 1992, 262(3): F462-F467.
[2] Lee H S, Park C B, Kim J M, et al. Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide[J]. Cancer letters, 2008, 271(1): 47-55.
[3] Scoltock A B, Cidlowski J A. Activation of intrinsic and extrinsic pathways in apoptotic signaling during UV-C-induced death of Jurkat cells: the role of caspase inhibition[J]. Experimental cell research, 2004, 297(1): 212-223.