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Product Data Sheet

KMG-301AM TFA

Cat. No.:HY-126220ACAS No.:1372642-78-8Molecular Formula: $C_{32}H_{28}F_3N_3O_8$ Molecular Weight:639.58

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light, stored under nitrogen

* In solvent: -80°C, 6 months; -20°C, 1 month (protect from light, stored under

nitrogen)

BIOLOGICAL ACTIVITY

Description

KMG-301AM TFA is the acetoxy methyl esterified form of KMG-301. KMG-301AM TFA successfully accumulates in mitochondria and then it is hydrolyzed to KMG-301. KMG-301 is an ${\rm Mg^{2^+}}$ -selective fluorescent probe functional in mitochondria in intact cells. Since the mitochondrial membrane is impermeable to KMG-301, it is not released upon depolarization of the mitochondrial membrane potential. KMG-301 can indicate changes in mitochondrial Mg2+ concentration and shows ${\rm Mg^{2^+}}$ transport across the mitochondrial membrane in the early phases of a cellular model^[1].

In Vitro

Experimental Protocols: This protocol only provides a guideline, and should be modified according to your specific needs^[1].

1. For optical imaging, $20 \mu M$ KMG-301AM TFA is applied to cells in Hanks' balanced salt solutions (HBSS) for 10 min on ice, so that hydrolysis of the acetoxymethyl ester by esterase present in the cytosol will be avoided.

 $HBSS\ contains\ (in\ mM):\ NaCl,\ 137;\ KCl,\ 5.4;\ CaCl_2,\ 1.3;\ MgCl_2,\ 0.5;\ MgSO_4,\ 0.4;\ Na_2HPO_4,\ 0.3;\ KH_2PO_4,\ 0.4;\ NaHCO_3,\ 4.2;\ D-glucose,\ 5.6;\ HEPES,\ 5\ (pH\ adjusted\ to\ 7.4\ with\ NaOH).$

- 2. Then, the cells are washed twice with HBSS and incubated for 15 min at 37° C to allow for complete hydrolysis of the acetoxymethyl ester form in mitochondria.
- 3. If you want the simultaneous use of KMG-104AM (HY-128536) and KMG-301AM TFA, cells are incubated with 5 μ M KMG-104AM in HBSS for 30 min at 37°C, and then stained with KMG-301AM TFA.
- 4. Isolated mitochondria are incubated with 20 μ M KMG-301AM TFA in Mitochondria Imaging Buffer (MIB) for 20 min at 37°C. Then, mitochondria are washed twice with MIB and further incubated for 15 min at 37°C.

MIB contains (in mM): KCl, 125; K_2HPO_4 , 2; $MgCl_2$, 1; HEPES, 5; EDTA, 0.02 (pH adjusted to 7.2 with KOH) or Mg^{2+} -free MIB (without $MgCl_2$)

5. Fluorescence imaging experiments are performed using a confocal laser scanning microscope system. KMG-301 is excited at 559 nm from a laser diode, and the signal was observed at 600-700 nm.

In the simultaneous measurement, KMG-104 and KMG-301 were excited at 488 nm from an Argon laser and 559 nm, respectively. Fluorescence was separated using a 560 nm dichroic mirror and observed at 500-545 nm and 600-700 nm, respectively.

 $\label{eq:mce} \mbox{MCE has not independently confirmed the accuracy of these methods. They are for reference only.}$

REFERENCES

[1]. Shindo Y, et al. Newly developed Mg²⁺-selective fluorescent probe enables visualization of Mg²⁺ dynamics in mitochondria. PLoS One. 2011;6(8):e23684.

 $\label{lem:caution:Product} \textbf{Caution: Product has not been fully validated for medical applications. For research use only.}$

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