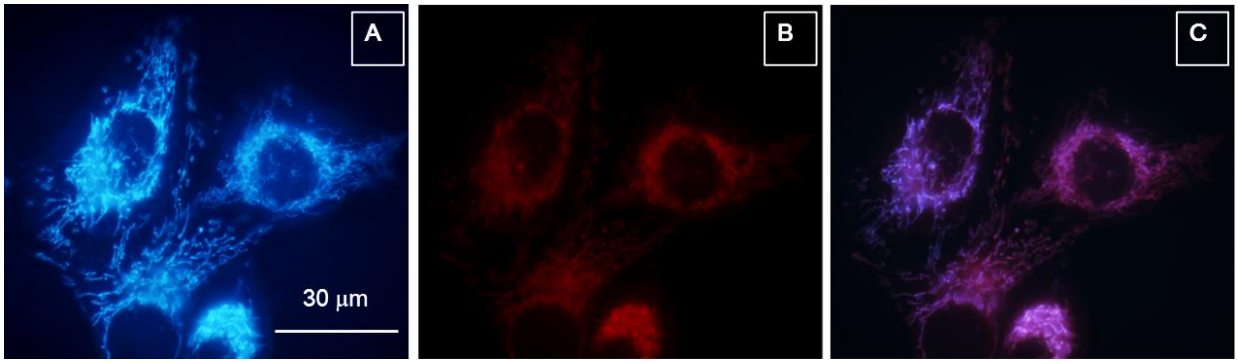
Product Specification

AIE™ Mitochondria Blue



Product Description

- This product can stain the mitochondria with blue fluorescence.
- After incubation with this product, cells can be observed under fluorescence microscope and blue signals can be obtained (Ex/Em=360/480 nm).
- This product can be co stained with yellow and red mitochondrial probes for a whole spectrum fluorescence.
- Also the product has superior photostability compared to other commercial mitochondrial stains.
  (Signals can be retained after 50 scans.)

Demonstrations

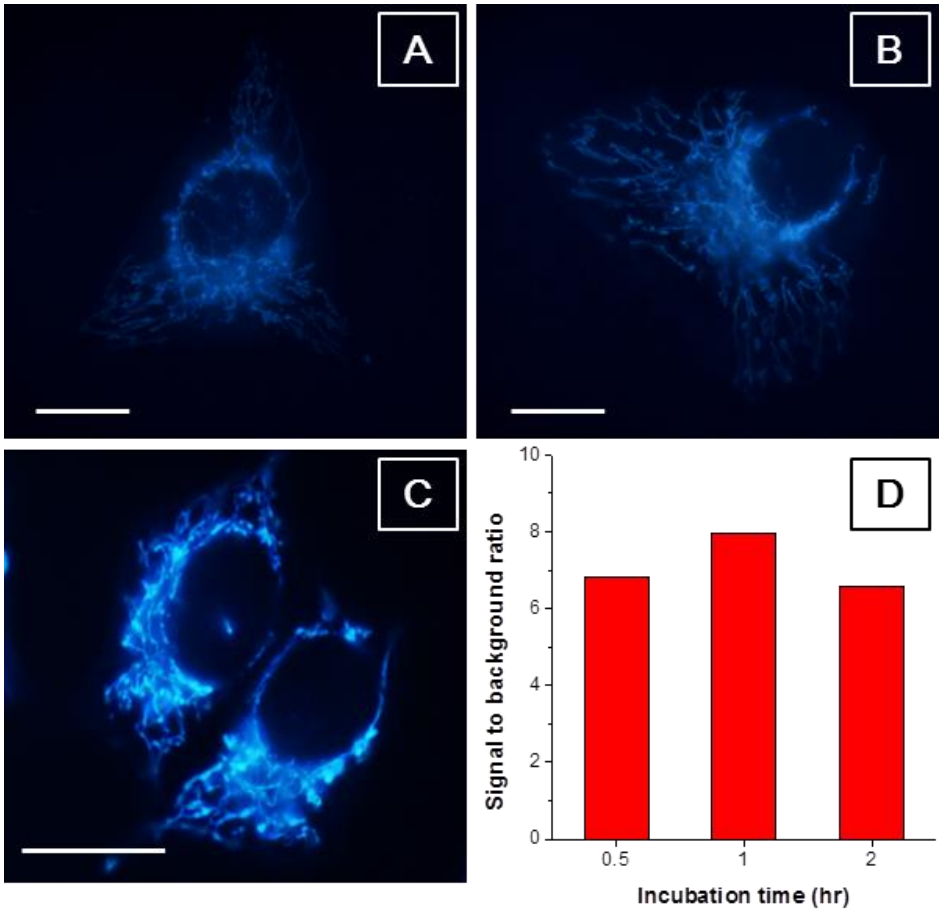


Figure 1. (A C) Fluorescence images of the HeLa cells stained with AIE™ Mitochondria Blue for (A) 30 min, (B) 1 h and (C) 2 h. (D) Signal to background ratio of AIE™ Mitochondria Blue dyed HeLa cells with different incubation time. Concentration of AIE™ Mitochondria Blue: 5 μM; excitation wavelength: 330-385 nm; scale bar: 15 μM.

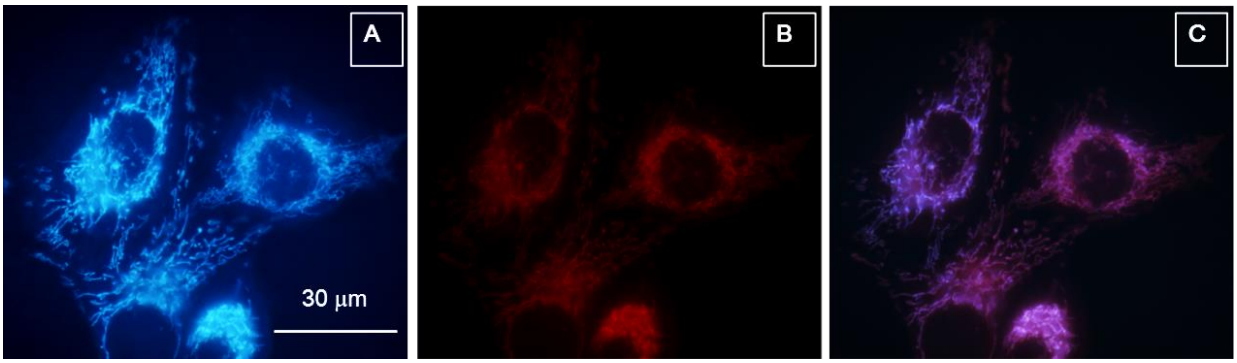


Figure 2. Fluorescence images of HeLa cells stained with (A) AIE™ Mitochondria Blue (5 μM) for 15 min and (B) Mitotracker Red FM (MT, 50 nM) for 1 h. (C) Merged images of A and B. Excitation wavelength: 330-385 nm for AIE™ Mitochondria Blue and 540-580 nm for MT.

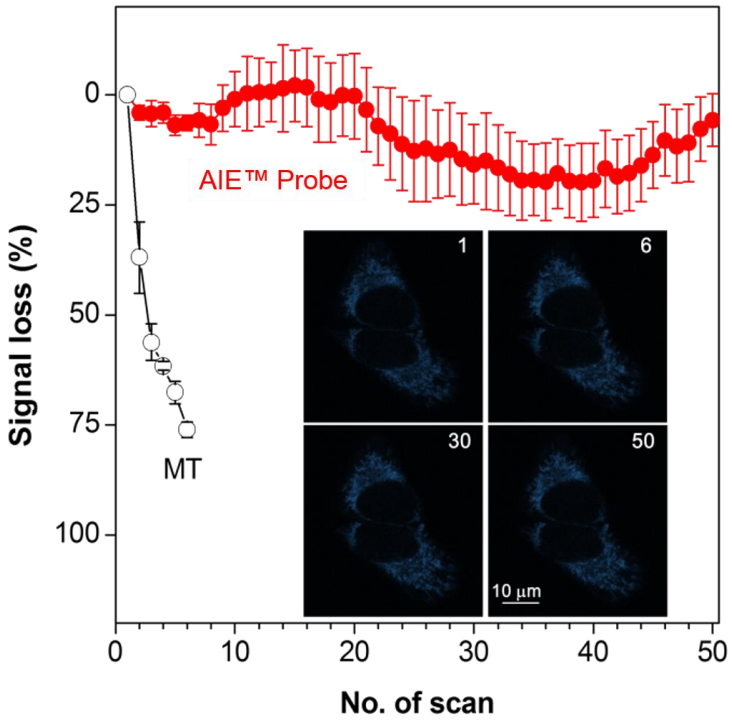


Figure 3. Fluorescence signal loss of AIE™ Mitochondria Blue (full) and MT (hollow) with increasing of number of scans. Insert: Fluorescent images of HeLa cells stained with AIE™ Mitochondria Blue (5 μM) after certain scans. Number of scan was noted on the right corner of each image. Excitation wavelength: 405 nm; emission wavelength: 449-520 nm, scanning time for each image: 7.75 s/scan.

Recommended storage condition

Store away from sunlight at 2-8 °C

Product parameters

Limited of Detection	**Mitochondria staining**
Color:	White powder
Imaging platform:	Fluorescence microscope Confocal microscope
Pack size and quantity:	10 μmol
Detection method:	Fluorescence
Excitation/Emission (nm):	360/480
Recommended transport condition:	Room temperature
Product declaration:	Only for research use.

AIEgen Probe for Mitochondria Targeting (Blue)

Introduction

- This product stains the mitochondria with blue fluorescence.
- After incubation with this product, cells can be observed under fluorescence microscope and blue signals can be obtained at following optical condition:

  Excitation / *Emission* = *360* ± *40* / *480* ± *50* nm
- This product can be co-stained with yellow and red mitochondrial probes for a whole spectrum fluorescence given that there is no FRET happen.
- The product has superior photostability compared to other commercial mitochondrial stains. Signals can be retained after 50 scans on confocal microscope with the 405 nm laser.

Material Preparation and Microscope Recommendation

- **Stock solution prepare:** AIE™ Mitochondria Blue (5 mM) stock solution is prepared with the 10 μmol of AIE™ Mitochondria Blue in 2 mL DMSO.
- **Fluorescence Microscope:** The HeLa cells could be imaged under a fluorescence microscope (λ_{ex} = 320 – 400 nm, diachronic mirror = 400 nm and emission filter = 420 nm long pass).

  # Note: Confocal Microscopy – Recommended with 405 nm laser as excitation (Power 6 %, at researcher's discretion).
- (Optional) The photostability test was investigated by confocal microscope. AIE™ Mitochondrial Blue was excited at 405 nm (6 % laser power) and the fluorescence was collected at 449 – 520 nm.

Before Your Experiment, You might NEED

1. Live cells
2. Culture media
3. Buffer PBS solution
4. DMSO
5. Milliphore water
6. 10 mM HEPES in MEM solution
7. (Optional) CCCP

Protocol (Recommended)

Cell Culture

The HeLa cells were cultured in minimum essential medium (MEM) containing 10 % fetal bovine serum and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5 % CO_2 humidity incubator at 37 ℃.

Cell Imaging

1. **Prepare:** HeLa cells were grown overnight on a petri dish (35 mm) with a coverslip.
2. **Staining**: The live cells were stained with 5 μM of AIE™ Mitochondrial Blue for 1 h (by adding 2 μL of a 5 mM stock solution in DMSO to 2 mL culture medium).
3. **Wash:** The live cells were washed with PBS three times after incubation with the product.
4. **Before imaging:** Imaging medium (10 mM HEPES in MEM) was added to the dish.
5. **Ready to go:** The cells were observed under a fluorescent microscope through the observation window.

Mitochondrial Morphological Change (Photostability Test)

1. HeLa cells were grown overnight on a plasma-treated 25 mm round coverslip mounted to the bottom of a 35 mm petri dish with an observation window.
2. The live cells were stained with 5 μM of the probe for 30 min (by adding 2 μL of a 5 mM stock solution of the probe in DMSO to 2 mL culture medium).
3. The live cells were washed with PBS three times after incubation with dyes.
4. 2 mL of imaging medium (10 mM HEPES in MEM) was added to the dish.
5. HeLa cells were then imaged by confocal microscope.
6. 10 μM CCCP (by adding 1 μL of a 200 mM stock solution of CCCP in DMSO to 2 mL culture medium) after the HeLa cells were imaged.
7. AIE™ Mitochondrial Blue-stained living HeLa cells were scanned with 7.75s / scan for 50 times.

Note

1. Drill a hole of around 10 mm diameter in the middle of the dish. Place cover slide over the dish using paraffin.
2. Add the CCCP carefully into the dish after imaging by confocal microscope as the disturbance to the cells should be minimized for comparing the morphological change.

Fluorescent Spectrum

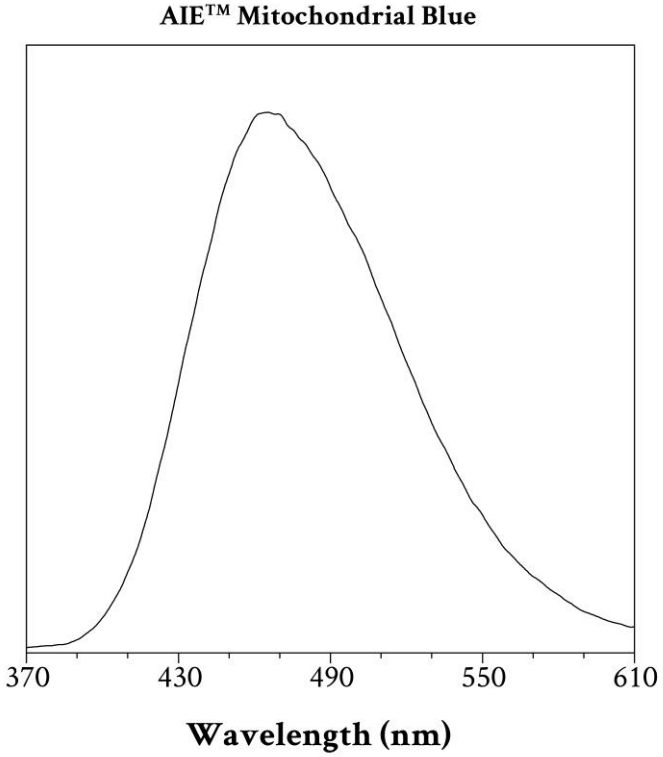


Figure 1 Photoluminescent spectrum of AIE™ Mitochondrial Blue probe in solid state. Excitation: 321 nm

Reference

1. Leung, C. W. T.; Hong, Y.; Chen, S.; Zhao, E.; Lam, J. W. Y.; Tang, B. Z. "A photostable AIE luminogen for specific mitochondrial imaging and tracking" *J. Am. Chem. Soc.*, **2013**, 35, 62–65.

2. Leung, C. W. T.; Hong, Y.; Tang, B. Z. "Specific imaging and tracking of mitochondria in live cells by a photostable AIE luminogen" *Advanced Protocols in Oxidative Stress III*, Methods in Molecular Biology Volume 1208, **2015**, pp 21–27

3. Optical information and suggested storage conditions:

Item	Ex/Em	Qty	Storage Condition*
AIE™ Mitochondria Blue	360 /480 nm	10 μmol	• ≤–20 ℃ (Upon receive this product) • Avoid Light • Keep Dry

* Remember to warm up to room temperature upon opening the vial