For research use only

# AcidiFluor™ ORANGE

Table 1. Product information

Code No.	Material	Amount	Storage	Stability
GC301	AcidiFluor™ ORANGE	10μg × 20 vials	Store under -20°C, desiccate and protect from light Unrecommend storing DMSO solution of dye	1 year (unopened)

### 1. Introduction

## ■ About *AcidiFluor™ ORANGE*

AcidiFluor™ ORANGE is a fluorescence imaging probe which shows strong fluorescence in acidic environments, but weak in neutral conditions. This probe can stain acidic organelles such as lysosome, late endosome and granule selectively. As it emits orange fluorescence by excitation at 532nm or 514 nm, it is suitable for multicolor imaging with blue fluorescence (CFP, Hoechst, etc.), green fluorescence (AcGFP, fluorescein, etc.) and near-infrared fluorescence. This probe can be used for long term timelaps imaging of acidic organelles, granule release, endocytosis/exocytosis and so on because of its good photostability.

## 2. Live cell staining protocol

#### ■ Materials Required but not Provided

- Anhydrous DMSO
- · Hank's Balanced Salt Solution (HBSS)
- •Cell culture medium

## ■ Preparation of Reagent and Cell Staining

- 1. To prepare a stock solution, dissolve the AcidiFluor™ ORANGE 10 µg in 13.4 µL of anhydrous DMSO to 1 mM.
- 2. Dilute the stock solution with medium to prepare 1 µM stain solution.
- 3. Remove the culture medium from cell culture dish and wash twice with medium.
  - Caution: Glass bottom dish etc. are recommended as cell culture dish, because it shows a low autofluorescence.
- 4. Add stain solution to the dish and incubate for 1 to 2 hours at 37°C, 5% CO<sub>2</sub>. Even though the degree of stained cells is varied with cell type or growth condition, usually cells are well stained around 2 hours.
- 5. After staining, wash once with medium and twice with HBSS buffer. Replace to HBSS buffer and observe the cells using a fluorescence microscopy.

# **■** Fluorescence Imaging

532 nm or 514 nm are suited for excitation wavelength. Cy3, TRITC (Nikon Co. Ltd.) or U-FGWA, U-FGWA, U-FGNA, U-FRF (Olympus Co. Ltd.) etc. are usable. The wavelength of maximum emission is around 560 nm.



## ■ Storage

Probes are forwarded under conditions of  $N_2$  atmosphere, dry and frozen state. After receipting, store under -20  $^{\circ}$ C, desiccate and protect from light.

Goryo Chemical Company commercialized AcidiFluor™ ORANGE under the guidance of Prof. Kenzo Hirose (Professor of Graduate School of Medicine, The University of Tokyo, Department of Neurobiology). AcidiFluor ORANGE™ was licensed from Tokyo University. The development of this product was supported by JST (Japan Science and Technology Agency) program "Development of Systems and Technology for Advanced Measurement and Analysis".

# ■ Related Products

Catalog no.	Product name	Description
GC302 GC303	AcidiFluor™ ORANGE- NHS	Acidic pH detecting probe.
GC304	AcidiFluor™ ORANGE- Labeling Kit	Ready-to-use labeling kit.
GC305	AcidiFluor™ ORANGE- Zymosan A	For imaging of phagocytosis.
GC306	AcidiFluor™ ORANGE- Dextran 10K	For imaging of endocytosis.
GC308	AcidiFluor™ ORANGE- wBeads500	Acidic pH indicating beads.
GC309	AcidiFluor™ ORANGE- Transferrin	For imaging of endocytosis.