GORYO CHEMICAL

For research use only

Updated: February 7, 2020

AcidiFluor™ ORANGE-NHS

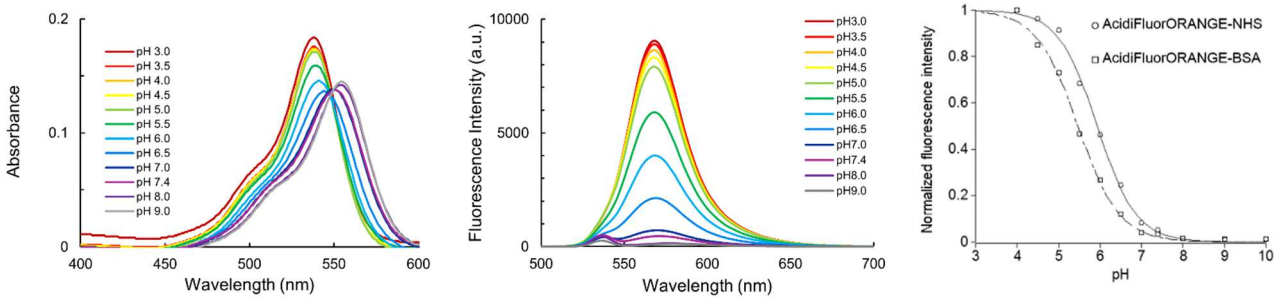
Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC302	AcidiFluor ORANGE-NHS	1 mg	≤–20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)
GC303		5 µg × 5		

1. About AcidiFluor ORANGE-NHS

AcidiFluor ORANGE-NHS is an acidic pH detecting probe which is used for protein labeling via N-hydroxysuccin-imide ester, just by mixing. Its fluorescent intensity increases ~20-fold at an acidic pH (Ex_{max} 544 nm, Em_{max} 565 nm, ε = 80,000 $M^{-1}cm^{-1}$, QY. = 0.7 at pH 3.0), compared to that in a physiological pH. It can be used to monitor pH changes along time, because of its reversibility of the fluorescence intensity upon pH changes, low cytotoxicity and the slow photobleaching.

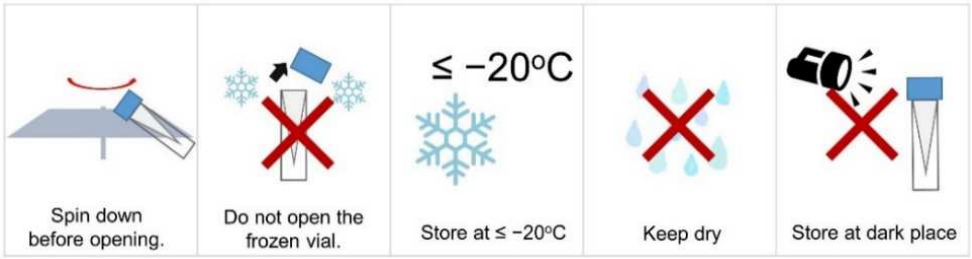
By using 1 mg vial of GC302, you could label 10-50 mg antibody protein, whereas by using 1 vial of GC303 (5 µg), you could label ~100 µg of antibody protein.



Absorption / fluorescence spectra of AcidiFluor ORANGE-NHS in each pH, and pH responsibility of BSA-conjugated reagent (λ_{ex} 532 nm/λ_{em} 568 nm.).

■ Storage

This product is shipped in a nitrogen gas-filled vial. Upon receipt, store the product desiccated and protected from light at ≤–20°C. Dissolve the solid just before use. Before opening the vial, spin down the solid to the bottom and warm the vial to the room temperature.



2. An example protocol for labeling antibody

■ Materials required but not provided

- 0.1 M sodium bicarbonate ($NaHCO_3$)
- Anhydrous DMSO
- Ultrafiltration column, gel filtration or dialysis tube with a suitable molecular size.

■ Preparation of reagent and fluorescence labeling

1. Dissolve the protein you wish to label in the 0.1 M $NaHCO_3$ buffer to 2-10 mg/mL. Use gel-filtration column to exchange the buffer if the protein is already dissolved in a neutral buffer solution. Refer the manufacture's instruction for the gel-filtration column.

   Note: Amine-containing reagents such as BSA, other protein, Tris, or glutathione inhibit the labeling reaction. In these cases, purify the protein before the labeling to remove the extra amines.
2. For **GC302** user, add 107 µL of DMSO to the 1 mg vial of AcidiFluor ORANGE-NHS, mix well to prepare 10 mM solution. Add this solution to the protein solution prepared in the previous step, at the molar ratio of 2 to 5. Immediately mix the solution by pipetting. For **GC303**, directly add the protein solution to the vial, and immediately mix the solution by pipetting to dissolve the AcidiFluor ORANGE-NHS solid.
3. Incubate the mixture at 25 ºC for 60 minutes. Protect from light during the incubation. Mix the solution by tapping the tube in every 15 minutes.
4. Remove unreacted dye by using gel-filtration column by exchanging buffer solution with PBS (pH 7.4).

■ Calculating degree of labeling

The degree of labeling (DOL) by AcidiFluor ORANGE-NHS can be calculated by the following equation:

$$\text{DOL} = \frac{A_{551}/\epsilon_{551,pH7.4}}{(A_{280}-A_{551}\times CF_{280/551})/\epsilon_{pr}},$$

Where the parameters represent as:

A_{551}, A_{280}: Absorbance of AcidiFluor ORANGE-conjugated protein at 551 nm and 280 nm, respectively

$CF_{280/551}$: A correction factor for the absorbance (see Table 2.)

$\epsilon_{551,pH7.4}$: An extinction coefficient of AcidiFluor ORANGE-NHS at 551 nm in pH 7.4 (see Table 2.)

ϵ_{pr}: An extinction coefficient of a protein at 280 nm. For IgG, 210,000 $M^{-1}cm^{-1}$.

Table 2. Properties of AcidiFluor ORANGE-NHS in pH 7.4

λ_{Abs} (nm)	$\varepsilon_{280,pH\ 7.4}$	$CF_{280/551}$
551	62,300	0.24

Note: The properties are for quantification of the dye in pH 7.4 PBS. These are different in acidic environment. At pH 3.0, it shows maximum absorption ε = 80,000 $M^{-1}cm^{-1}$ at 544 nm.

■ Fluorescence detection

For laser excitation, either 532 nm or 514 nm is appropriate. Fluorescence around 565 nm will be detected. For fluorescent microscopy, general green excitation filter set such as that for Cy3 can be used.

Table 3. Related Products

Catalog no.	Product name	Major applications
GC301	AcidiFluor ORANGE	For imaging of lysosome
GC304	AcidiFluor ORANGE Labeling Kit	All-in-one package for antibody labeling.
GC305	AcidiFluor ORANGE-Zymosan A	For the detection of phagocytosis.
GC306	AcidiFluor ORANGE-Dextran 10k	For the detection of endocytosis.
GC309	AcidiFluor ORANGE-Transferrin	For the detection of endocytosis.
GC310-01	HaloTag® AcidiFluor ORANGE ligand	Acidic pH indicator conjugated with HaloTag® ligand.

GORYO Chemical, Inc.
EAREE Bldg. 5F, Kita 8 Nishi 18-35-100, Chuo-ku, Sapporo 060-0008, JAPAN
TEL: +81-11-624-5860 https://goryochemical.com/