GORYO CHEMICAL

For research use only

Updated: February 7, 2020

AcidiFluor™ ORANGE Labeling Kit

Table 1. Product information

Catalog no.	Product name	This kit includes		Storage upon receipt	Stability
GC304	AcidiFluor ORANGE Labeling Kit	AcidiFluor ORANGE-NHS Reaction buffer Washing buffer Ultrafiltration spin column	5 vials 1.5 mL×1 10 mL×1 5 tubes	≤–20°C, keep desiccated and protected from light.	1 year (when unopened)

1. About AcidiFluor ORANGE Labeling Kit

This product is an antibody-labeling kit of acidic-pH detecting fluorescent probe. Its orange fluorescence intensity in acidic organelles (pH ~5.0) is ~20 times stronger than that in the physiological pH 7.4. It shows maximum fluorescence intensity (Ex_{max} 544 nm, Em_{max} 565 nm, ε = 80,000 $M^{-1}cm^{-1}$, QY. = 0.7) in solution conditions at pH ≤3.0. It is suitable to detect the time-dependent changes of intracellular pH changes because of the reversibility of the fluorescence upon pH, low cytotoxicity, and high photostability. This kit includes all reagents and disposable apparatus required for the labeling and the purification.

※ Using one vial of AcidiFluor ORANGE-NHS, 100 µg of antibody (IgG, MW = 150 kDa) can be labeled.

※ Besides of antibodies, proteins or other molecules of >30 kDa which has primary amines can be also labeled.

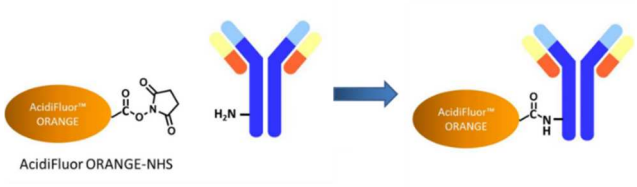


Figure 1. The reaction between AcidiFluor ORANGE-NHS and antibody

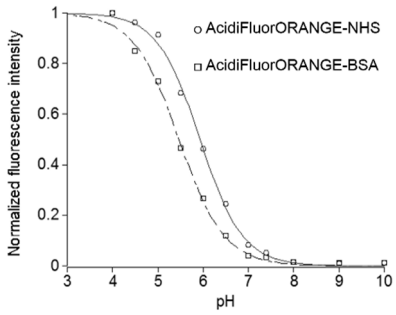
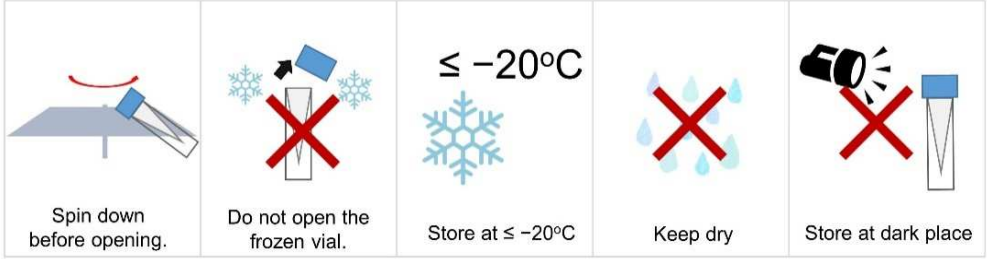


Figure 2. pH-dependent fluorescent intensity of AcidiFluor ORANGE-NHS and AcidiFluor ORANGE-conjugated BSA. λ_{ex} 532 nm/λ_{em} 568 nm

■ Storage

This product is shipped in a nitrogen-gas filled vial. Upon receipt, store the product desiccated and protected from light at ≤ –20°C. Storing as a solution is not recommended.



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2. A protocol for protein labeling

■ Materials required but not provided

- Micropipettes and pipette tips.
- Microcentrifuge
- Microvolume-spectrophotometer (eg. Thermo Scientific™ NanoDrop™)
- Antibody you wish to label. *If the antibody is dissolved in Tris-buffer, BSA containing buffer solution, or other solution including amine-containing reagents, purification to remove the amine-containing substances is required before the labeling.*
- Safety eyeware, disposable groves, and a labware.

■ Preparation of reagent and fluorescent labeling method

1. Leave the kit components in dark until they recover to the room temperature completely before opening the caps.
2. Rinse the membrane filter by adding 200 µL of the Washing Buffer to the Ultrafiltration spin column and centrifuging at 5,000 ×*g* for 10 minutes. Discard the filtrate.
3. Dissolve 100 µg of the antibody in 100 µL of the Reaction Buffer, if the antibody is dry powder. If it is already in a buffer solution, put the solution on the Ultrafiltration spin column and centrifuge at 5,000 ×*g* for 3-10 minutes until the volume to be <20 µL. Then mess it up to 200 µL by adding the Reaction Buffer, mix well by pipetting, centrifuge at 5,000 ×*g* for 3-5 minutes until the volume to be 100 µL, and mix well again.
4. Spin down the vial of the AcidiFluor ORANGE-NHS, open the cap and add the protein solution prepared at step 3 to the vial. Mix well by pipetting up and down. Incubate the reaction mixture over 1 hour at room temperature. During the reaction, the reaction mixture should be protected from light. Keep the spin column if you used one at step 3.
5. Transfer the reaction mixture to the ultrafiltration spin column prepared at step 2 or used at step 3.
6. Add another Washing Buffer 100 µL and centrifuge at 5,000 ×*g* for 10 minutes. If the solution remains on the membrane, centrifuge for 5 minutes or more.
7. Discard the filtrate and repeat the step 6 for three times.
8. Add Washing Buffer 100 µL to the ultrafiltration column and recover the labeled protein on the membrane. Transfer the protein solution to a new microtube and preserve at 4°C.

■ Calculate the degree of labeling

You can calculate the degree of labeling (DOL) by the following equation:

$$\mathrm{DOL} = \frac{A_{551}/\varepsilon_{\mathrm{AcidiFluor}}}{(A_{280} - A_{551} \times \mathrm{CF})/\varepsilon_{\mathrm{Protein}}}$$

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

CF: Correction Factor （See Table 2.）

$\varepsilon_{\mathrm{AcidiFluor}}$: Extinction coefficient of AcidiFluor ORANGE-NHS (See Table 2.)

$\varepsilon_{\mathrm{Protein}}$: Extinction coefficient of a protein. It is 210,000 for IgG.

Table 2. Properties of AcidiFluor ORANGE-NHS

λ_{Abs} (nm)	ε	CF
551 nm	62,300	0.24

※ Properties of AcidiFluor ORANGE-NHS changes upon the solution pH. The DOL in PBS pH 7.4 can be calculated using the above parameters. On the other hands, the properties changes in acidic environments.

■ Fluorescent observation

532 nm or 514 nm are suited for excitation wavelength. Cy3, TRITC (Nikon) or U-FGWA, U-FGW, U-FGNA, U-FRF (Olympus) filter sets are recommended. The wavelength of maximum emission is approximately 560 nm.

■ References

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

Table 3. Related Products

Catalog no.	Product name	Major applications
GC301	AcidiFluor ORANGE	For imaging of lysosome
GC302	AcidiFluor ORANGE-NHS	For labeling of antibodies or other proteins
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.

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