

For research use only

HaloTag[®] AcidiFluor[™] ORANGE Ligand

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC310-01	HaloTag [®] AcidiFluor [™] ORANGE Ligand	30 nmol	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)
GC310-02		60 nmol		

1. About HaloTag[®] AcidiFluor[™] ORANGE Ligand

AcidiFluor[™] ORANGE is an acidic pH indicator which shows almost no fluorescence in neutral pH but emits orange fluorescence in acidic environments. Its fluorescence intensity reversibly modulated upon pH changes of the surrounding medium. This product is conjugated to chloroalkane which rapidly form covalent bond with HaloTag[®]. It enables specific and stable labeling of HaloTag[®] conjugated proteins just by mixing and then monitoring pH changes. Relatively photostable fluorescence of AcidiFluor[™] ORANGE enables time lapse imaging of pH change and tracking of endocytic/exocytic vesicles for long-time period.

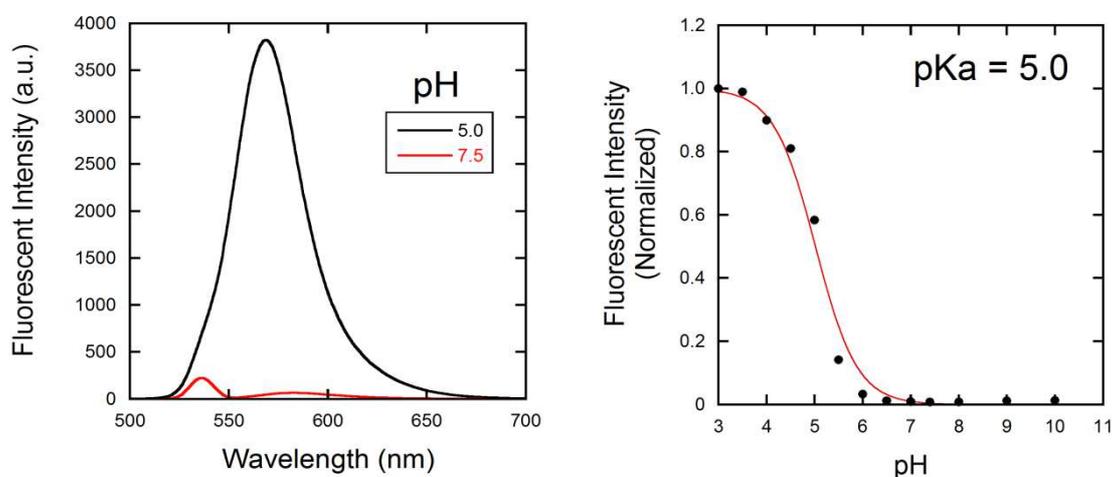


Figure 1. (left) Fluorescence emission

spectra in pH 5.0/7.5 buffer solution.

(right) Fluorescence intensity changes upon pH of the solution.

Table 2. Product specifications

λ_{ex} (nm)	λ_{em} (nm)	ϵ ($\text{M}^{-1} \text{cm}^{-1}$)	Φ_{max}
520	565	60,000	0.7

■ Storage

This product is shipped as a dried powder 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.

2. An example of live cell imaging

■ Preparation of reagent

- ① HaloTag® AcidiFluor™ ORANGE Ligand is purple solid. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
- ② Warm the vial to the room temperature and add 30 µl (GC310-01) or 60 µl (GC310-02) of purified (double-distilled) water to one vial to prepare 1 mM stock solution. Note that the reagent is not stable in dimethylsulfoxide (DMSO). Dissolve the solid completely by careful pipetting. Solution will be a red purple liquid.

■ Example cell imaging procedure

- ① Prepare cells expressing HaloTagged recombinant protein which will be incorporated in acidic organelles (ex. VAMP2, or EGFR). Seed the cells on a glass-bottom dish and cultured for >12 hrs at 37°C, 5% CO₂.
- ② Dilute the 1 mM of HaloTag® AcidiFluor™ ORANGE Ligand solution with culture medium to prepare 10 µM solution. Keep the solution at 37°C.
- ③ Add 1/5 volume of the cell culture medium in the dish to make final 2 µM of HaloTag® AcidiFluor™ ORANGE Ligand. Incubate at 37°C, 5% CO₂ for 15 minutes.
- ④ After staining, rinse cells with observation buffer or culture medium without phenol red to remove free fluorescent probe.

■ Fluorescent observation

Observe cells by fluorescent microscopy using green excitation filter set such as G-2A, Cy3, TRITC (Nikon) or U-FGWA, U-FGW, U-FGNA, U-FRFP (Olympus). Alternatively, use 532 or 514 nm laser for excitation and observe emission around 560 nm. This probe can be also excited with 488 nm laser, however, the excitation efficiency is not high.

■ Reference

D. Asanuma, Y. Takaoka, S. Namiki, K. Takikawa, M. Kamiya, T. Nagano, Y. Urano & K. Hirose. (2014) Acidic-pH-Activatable Fluorescence Probes for Visualizing Exocytosis Dynamics. *Angew. Chem. Int. Ed.* **53**: 6085–6089.

Table 2. Related Products

Catalog no.	Product name	Major applications
GC301	AcidiFluor™ ORANGE	For staining lysosomes of cells without expressing tags.
GC302	AcidiFluor™ ORANGE-NHS	To label primary amines (proteins) via NHS.
GC305	AcidiFluor™ ORANGE-Zymosan A	For imaging phagocytosis.
GC306	AcidiFluor™ ORANGE-Dextran 10K	For imaging endocytosis.
GC308	AcidiFluor™ ORANGE-wBeads500	Acidic pH indicating beads.
GC309	AcidiFluor™ ORANGE-Transferrin	For imaging endocytosis.
A201-01	HaloTag® HMSiR Ligand	For superresolution imaging of HaloTag® proteins.
A308-01	HaloTag® STELLA Fluor™ 650 Ligand	For imaging HaloTag® protein in near-infrared wavelength.

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