

AcidiFluor™ ORANGE-Zymosan A

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC305	AcidiFluor ORANGE-Zymosan A	1 mg	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About AcidiFluor ORANGE-Zymosan A

Zymosan A is a compound prepared from yeast cell walls and often used to induce inflammatory responses including phagocytosis. AcidiFluor ORANGE-Zymosan A is Zymosan A labeled with AcidiFluor ORANGE, an acidic pH indicator. Its fluorescence greatly enhances in the acidic environment including phagosomes, therefore AcidiFluor ORANGE-Zymosan A is a useful probe to detect phagocytosis via its fluorescence increase which can be only detected after phagocytosis. It can be applied

for flow cytometric analysis and time-lapse imaging of phagocytosis.

Table 2. Photophysical properties of the fluorophore

Ex_{max} (nm)	Em_{max} (nm)	pKa	ϵ
535	568	5.8, 7.0	80 000

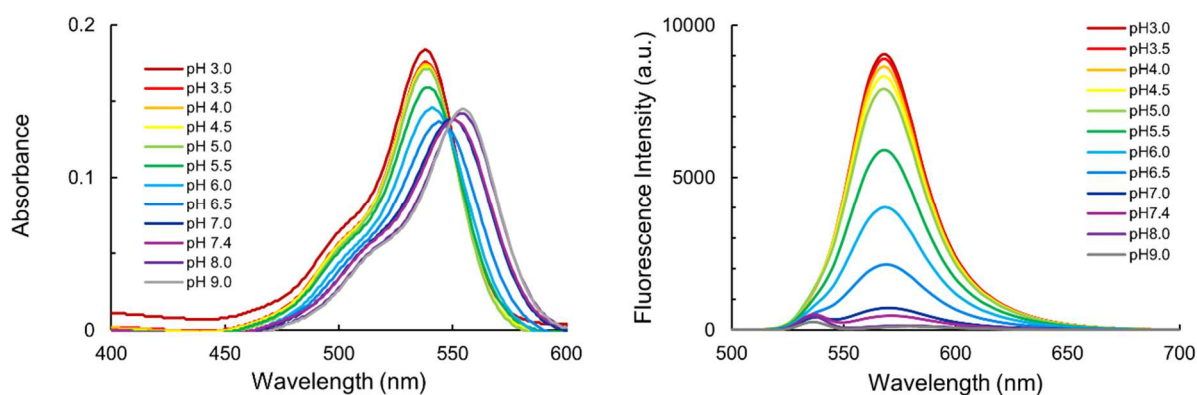
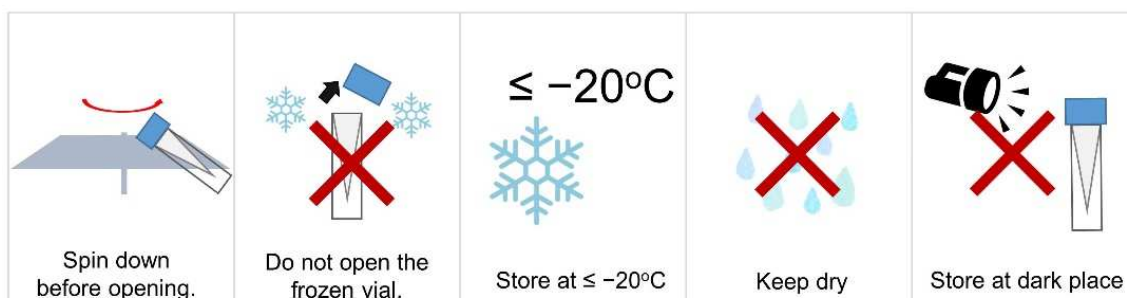


Figure 1. Spectra of the fluorophore. Fluorescence spectra measured with 535-nm excitation.

■ Storage

Store the product desiccated and protected from light at ≤ -20°C. Storing as a solution is not recommended.



2. Protocols

■ Preparation of reagent

1. Suspend the AcidiFluor ORANGE-Zymosan A with an appropriate cell culture medium. This reagent is not soluble to aqueous media.
2. Sonicate the suspension using a bath sonicator for two minutes. Then cool the suspension on an ice bath for two minutes. Repeat the sonication/cooling cycle for five times to uniformly disperse the suspension.

■ Example procedures

Imaging of phagocytosis by RAW264.7 cells

1. Seed RAW264.7 cells to a glass-bottom dish and culture the cells for overnight.
2. Remove cell culture medium and add AcidiFluor ORANGE-Zymosan A suspension of 10 µg/mL in an appropriate culture medium (e.g. DMEM, 10% FBS, penicillin, and streptomycin). Incubate the cells at 37°C, 5% CO₂ for 1–2 hours. In usual conditions, 1 hour incubation is enough.
3. Remove the cell culture medium including AcidiFluor ORANGE-Zymosan A, then, rinse cells with a washing buffer such as Hank's balanced salt solution (HBSS) twice to remove extra AcidiFluor ORANGE-Zymosan A. Gentle handling not to detach the cells from the dish is required.
4. Replace the solution with an observation solution such as HBSS or a culture medium without phenol red, and observe the cells with fluorescence microscopy.

※ Optimizations of the AcidiFluor ORANGE-Zymosan A concentration is recommended. In a test at Goryo Chemical Inc., ~10% of the cells were fluorescently positive when 10 µg/mL of the reagent had been added; ~20% positive cells were observed when 20 µg/mL of the reagent had been added; ~50% positive cells were observed when 50 µg/mL of the reagent had been added. On the other hand, the larger amount of the reagent, the more extra Zymosan A will be on the transmitted light images.

Flow cytometric analysis of phagocytosis

1. Prepare conditioned RAW264.7 cells attached to a cell culture dish.
2. Remove cell culture medium and add 50 µg/mL AcidiFluor ORANGE-Zymosan A suspended in an appropriate cell culture medium. Incubate cells at 37°C, 5% CO₂ for 1–2 hours. In usual conditions, 1 hour incubation is enough.
3. Remove the cell culture medium including AcidiFluor ORANGE-Zymosan A. Rinse cells with a washing buffer such as HBSS twice to remove extra AcidiFluor ORANGE-Zymosan A. Gentle handling not to detach the cells from the dish is required.
4. Add Trypsin-EDTA to detach cells from the dish. Add cell culture medium including serum to neutralize the trypsin. Then separate cells from the medium using a centrifuge.
5. After the removal of the cell culture medium, suspend cells with PBS, and remove debris using a cell strainer.
6. Analyze the cells with a flow cytometer.

■ Fluorescence detection

AcidiFluor ORANGE is well excited with 532 nm green laser. Alternatively 488-nm laser can be also used. In fluorescence microscopy, green excitation filter set such as that for Cy3 is appropriate. For flow cytometric analysis, a fluorescence filter for phycoerythrin is suitable.

Table 2. 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.
GC304	AcidiFluor ORANGE labeling kit	All-in-one package for antibody labeling.
GC306	AcidiFluor ORANGE-Dextran 10k	For the detection of endocytosis.
GC307	AcidiFluor ORANGE-Beads	AcidiFluor ORANGE conjugated with silica microparticles.
GC308	AcidiFluor ORANGE-wBeads	Dual-color pH sensing particles which composed of FITC.
GC309	AcidiFluor ORANGE-Transferrin	For the detection of endocytosis.
GC310-01	HaloTag® AcidiFluor ORANGE Ligand	Acidic pH indicator conjugated with HaloTag ligand.
GC3006-01	HySOx	For the detection of hypochlorous acid.