

For research use only

AcidiFluor ORANGE™-NHS

Table 1. Product information

Code no.	Product	amount	Storage	Stability	MW
GC302	AcidiFluor ORANGE™-NHS	1 mg	Store under -20 °C, desiccate and protect from light. Unrecommend storing DMSO solution of dye	1 year (unopened)	935.74
GC303	AcidiFluor ORANGE™-NHS	5 samples			

1. Introduction

■ About AcidiFluor ORANGE -NHS

AcidiFluor ORANGE™-NHS (succinimidyl ester) is amine reactive pH probe. Protein samples and terminal aminated oligonucleotide samples can be labeled by AcidiFluor ORANGE –NHS only by mixing.

【GC302】 1 mg of AcidiFluor ORANGE™ -NHS can label about 10 mg -50 mg proteins.

【GC303】 1 vial of AcidiFluor ORANGE™ -NHS can label about 100 µg of IgG.

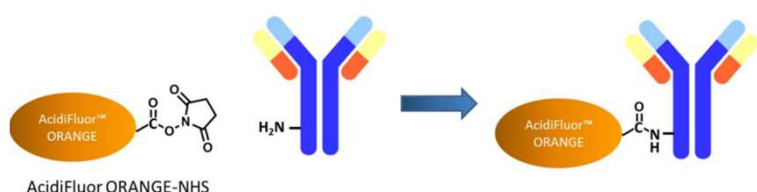


Fig 1. Reaction of AcidiFluor ORANGE™-NHS and antibody

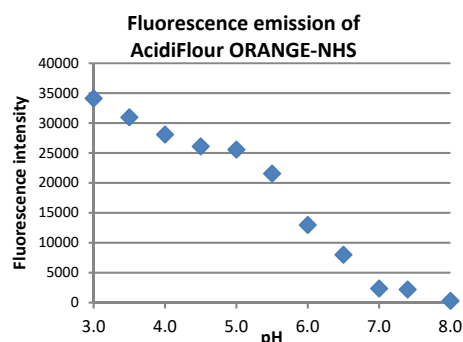


Fig 2. pH dependent fluorescence spectrum of AcidiFluor ORANGE™-NHS

2. Protein labeling protocol

■ Materials Required but not Provided

- 0.1 M sodium bicarbonate buffer (pH 8.3)
- Ultrafiltration column, gel filtration or dialysis tube with a suitable molecular size.

■ Preparation of Reagent and fluorescence labeling method

- ① Dissolve the protein in 0.1M sodium bicarbonate buffer(pH 8.3) to 2 -10 mg/mL.

Note: Amine-containing reagents such as BSA, other protein, Tris, glutathione, etc. inhibit labeling reaction.

To label such samples, purification prior to the labeling is required.

- ② Dissolve AcidiFluor ORANGE™-NHS in H₂O (deionized water) to 10 mg/mL and mix well.

- ③ Add 20 μ L of AcidiFluor ORANGE™-NHS solution (②) slowly to 1 mL of the protein solution (①), and stir over 1 hour at room temperature. During the reaction, the reaction mixture should be protected from light.

Note; In the case of GC303, 100 μ L of protein solution and 5 μ L of AcidiFluor ORANGE™-NHS solution are mixed.

- ④ We recommend using PALL nanosep® 10K omega membrane centrifugal device to remove the unreacted AcidiFluor ORANGE™-NHS.

■ Confirmation of conjugate

You can observe the conjugate by SDS-PAGE analysis.

3. Calculate the Labeling ratio

The degree of labeled protein by AcidiFluor ORANGE™-NHS can be calculate as following equation,

$$\text{Labeling Ratio} = \frac{A_{551} / \epsilon_{\text{AcidiFluor ORANGE-NHS}}}{(A_{280} - A_{551} \times \text{CF}) / \epsilon_{\text{protein}}}$$

$A_{551, 280}$: Absorbance of AcidiFluor ORANGE-protein in 551 nm, 280 nm

CF : Correction Factor (see Table 2)

$\epsilon_{\text{AcidiFluor ORANGE-NHS}}$: Extinction coefficient of AcidiFluor ORANGE™-NHS (see Table 2)

$\epsilon_{\text{protein}}$: Extinction coefficient of protein

in case of IgG: 216,000

Table 2. The Properties of AcidiFluor ORANGE™-NHS

	pK _a	Abs max	Flu max	ϵ	CF (Correction Factor)
AcidiFluor ORANGE™-NHS	5.3, 6.8	551 nm	571 nm	62,300	0.24