GORYO CHEMICAL

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For research use only

POLARIC® Labeling Kit

Table 1. Product information

Code No.	Material	Contents	Storage	Stability
GC211	***POLARIC® Labeling Kit***	・POLARIC-NHS ×5 ・Dimethylsulfoxide 500 µL ×1 ・Reaction Buffer 1.5 mL ×1 ・Washing Buffer 10 mL ×1 ・Ultrafiltration column ×5	Store under -20 deg. C, desiccate and protect from light Use up at once after probes are solved to DMSO	1 year (unopened)

1. Introduction

■ About amino reactive probe

POLARIC-NHS (succinimidyl ester) is pH probe for labeling protein and nucleic acid, and not need condensing agent. Not only proteins but terminal aminated oligonucleotide can be labeled by POLARIC –NHS.

The contents of this kit cover from labeling to purification. A portion of POLARIC-NHS is included most appropriate amount for labeling 100 µg of IgG.

CAUTION: DO NOT use for 30kDa or fewer samples.

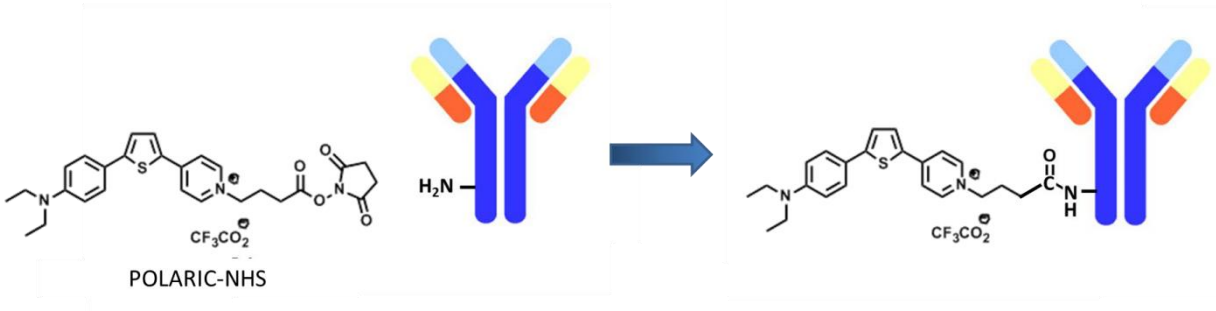

Fig 1. Reaction of POLARIC-NHS and antibody

2. Protein labeling protocol

■Materials Required but not Provided

・Microtubes for reaction and preservation

・Micropipetter

・Microcentrifuge

■Preparation of Reagent and fluorescence labeling method

① Rinse the membrane filter; add Washing Buffer 200 µL to ultrafiltration column and centrifuge at 5,000 *g* for 10 minutes. Discard the filtrate.

② Dissolve the 100 µg of protein in 100 µL of Reaction Buffer.
If BSA, other protein, Tris or glutathione, etc. are contained in target protein, these substance inhibit protein labeling. Protein should be purified prior to labeling reaction

③ Immediately before the reaction, dissolve POLARIC™-NHS in 10 µL of DMSO and mix well.
Because amino reactive probes are unstable in DMSO, you should go to the next step.

④ 100 µL of the protein solution (②) in 10 µL of POLARIC™-NHS solution (③), and pipetting up and down then react at room temperature, protect from light for over an hour.

⑤ Add all of reacted solution (④) to ultrafiltration column.

⑥ Add another Washing Buffer 100 µL and centrifuge at 5,000 *g* for 10 minutes. If solution is remained on membrane, centrifuge additional 5 minutes or more.

⑦ Discard the filtrate and repeat the operation ⑥.

⑧ Add Washing Buffer 100 µL to ultrafiltration column and recover the labeled protein on the membrane. Transfer the protein solution to new microtube and preserve at 4 deg. C.

■Confirmation of conjugate

Available for UV (~350 nm) observation after SDS-PAGE.

3. Calculate the Labeling ratio

The number of bound POLARIC-NHS per 1 molecule of protein can be calculate as below,

$$\text{Labeling Ratio} = \frac{A_{480} / \varepsilon_{\text{POLARIC-NHS}}}{(A_{280} - A_{480} \times \text{CF}) / \varepsilon_{\text{protein}}}$$

$A_{480,\ 280}$ ： Absorbance of POLARIC-protein in 480 nm, 280 nm

CF ： Correction Factor (see Table 2)

$\varepsilon_{\text{POLARIC-NHS}}$ ： Extinction coeffient of POLARIC-NHS (see Table 2)

$\varepsilon_{\text{protein}}$ ：Extinction coeffient of protein

in case of IgG: 216,000

Table 2. Properties of POLARIC-NHS

	Abs max	Flu max	ε	CF (Correction Factor)
POLARIC®-NHS	480 nm	554 nm*	50,000	0.31

* This value measured in aqueous solution.
Solvatochromic fluorophore POLARIC is changed by its environment.

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