We recommend using the following general protocol for the labeling of biomolecules with NHS esters

1. Calculate required amount of NHS ester:

NHS_ester_weight [mg] = 8 × amino_compound_weight [mg] × NHS_ester_molar_weight [Da] / amino_compound_molar_weight [Da].

8 is molar excess of NHS ester. It is experimental value for mono-labeling, suitable for many common proteins and peptides. However, in some cases using less or more NHS ester is required. It depends on protein structure, reagent, and solubility. Molar weight of APExBIO products can be found on corresponding product pages.

For example, to label 3 mg of BSA (molar weight 69300 Dalton) with Cy5 NHS ester (molar weight 616 Dalton), and obtain maximum yield of mono-labeled product, one should use
8 × 3 mg × 616 Da / 69300 Da = 0.21 mg
of Cy5 dye NHS ester.

2. Determine volume of reaction mixture. The labeling can be performed on any scale from nanomols to dozens of grams. When the scale is low, use minimal volume (10-20 uL). Higher concentrations (1-10 mg of amino-biomolecule per mL of mixture) are optimal.

3. Dissolve NHS ester in 1/10 reaction volume of DMF or DMSO. Amine-free DMF is preferred solvent. After the reaction, NHS ester can be stored in solution for 1-2 months at -20 ℃.

4. Dissolve biomolecule in 9/10 reaction volume of buffer with pH 8.3-8.5.

0.1 M Sodium bicarbonate solution has appropriate pH. Another alternative is 0.1 M phosphate buffer. Note pH is the most important thing. Avoid using buffers containing amines (Tris can sometimes be used but not recommended).

When doing large-scale labeling (hundreds of milligrams of NHS ester), note that the mixture tends to acidify with time because of hydrolysis of NHS ester. Monitor pH, or use more concentrated buffer then.

5. Add NHS ester solution to the solution of biomolecule, and vortex well. Keep on ice overnight, or at room temperature during at least 4 hours.

6. Purify the conjugate using appropriate method: gel-filtration for macromolecules is most universal. Precipitation and chromatography is another alternative. Organic impurities (such as N-hydroxysuccinimide, NHS ester, acid produced by hydrolysis) are almost always easily separated. For proteins and nucleic acids, ethanol or acetone precipitation can be used.