Reaction Conditions for Chemical Coupling (S9148)

Overview

We recommend that coupling reactions between BG-NH_2 and NHS esters take place in anhydrous N,N-dimethyl formamide (DMF) using triethylamine as a base.

Coupling reactions typically use an excess of the BG-NH_2 (1.5 equivalents) relative to the desired NHS label (1 equivalent) at 5–20 mM final concentration in the presence of a 1.5-fold molar excess of triethylamine in DMF. The reaction is generally performed at 30°C overnight.

Example Reaction: Coupling of BG-NH_2 to Alexa Fluor® 350 NHS-ester:

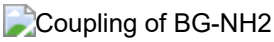

Figure 1. Coupling of BG-NH_2 to Alexa Fluor® 350 NHS ester. A solution of 2.0 mg (4.9 µmol) Alexa 350 NHS-ester in 500 µl DMF was added to 2.0 mg (7.4 µmol) BG-NH_2 and 1.1 µl triethylamine (7.9 µmol) in 500 µl DMF. The reaction was shaken at 30°C overnight. The solvent was evaporated under vacuum. The reaction was diluted in 1 ml water/acetonitrile (9:1) and the product purified by reverse phase HPLC.

The purification strategy will depend on the label used and the reaction scale. Good results have been obtained with both HPLC and silica gel chromatography.