

Bio-P1: Ligating the peptide to the C-terminus of a thioester-tagged protein

Overview

Protocol

1. Combine 10 μ l of the resuspended Bio-P1 peptide with 40 μ l of protein purified using the IMPACT-TWIN or IMPACT-CN Systems. It is important to use a C-terminal intein fusion and MESNA to induce intein cleavage during protein purification (see the FAQs pertaining to IPL in the IMPACT Kits). We recommend incubating the Bio-P1 peptide and thioester-tagged protein overnight at room temperature. If the target protein is unstable at room temperature, then use an overnight incubation at 4°C. If the unreacted Bio-P1 peptide interferes with subsequent experimentation it can be separated from the protein by FPLC using a column appropriate for the target protein.