Intein-Mediated Protein Ligation (IPL) and Labeling with the IMPACT™ Kit

Introduction

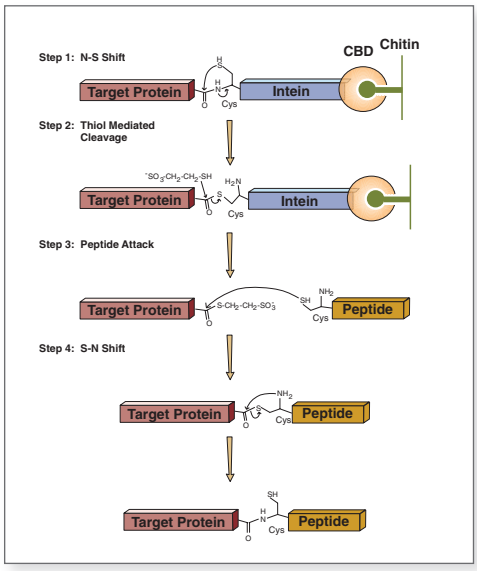
The IPL reaction, also referred to as expressed protein ligation, allows the ligation of a bacterially expressed protein or a synthetic peptide with an N-terminal cysteine residue to a protein with a C-terminal thioester through a native peptide bond (Figure 1; 1,2). In addition to protein purification, pTXB, and other C-terminal fusion vectors (pTYB and pTWIN series) used with the IMPACT™ Kit (NEB #E6901) can generate a protein with a C-terminal thioester for IPL. Typically 2-mercaptoethanesulfonic acid (MESNA) is used as the thiol reagent to induce intein-mediated cleavage; this produces a C-terminal thioester on the target protein. The C-terminus of the target protein can then be covalently labeled or ligated to a synthetic peptide with an N-terminal cysteine.

General Protocol

1. One of the components should have a final concentration of at least 0.5–1 mM. For the ligation of a peptide to a protein we use 0.01–0.1 mM protein with 0.5–1 mM peptide.
2. Combine the two components in the presence of 0.1 M Tris, pH 8.5, 0.1–0.5 M NaCl and 10 mM MESNA and incubate overnight at 4°C. Alternatively, the reaction can be incubated at 25°C for 1–4 hours.
3. The ligation may be visualized by a 10% or 12% SDS-PAGE as a shift in mobility of the ligated protein, or can easily be detected by Western blot using an antibody specific for the peptide. Add 3X SDS Sample Buffer (with DTT) to the protein sample, boil for 5 minutes and analyze by SDS-PAGE, with unligated protein as a control (Figure 2).

To label a protein with biotin, a peptide with an N-terminal cysteine and a biotinylated residue can be used. The biotinylated protein sample can be analyzed by SDS-PAGE and detected by Western blot with anti-biotin antibody. Alternatively, a protein can be labeled with a peptide posessing an N-terminal cysteine and a fluorophore conjugated to a residue. Though a purified peptide is not required it can usually yield a higher ligation efficiency (75%–90%).

Figure 1: Mechanism of intein-mediated protein ligation (IPL)



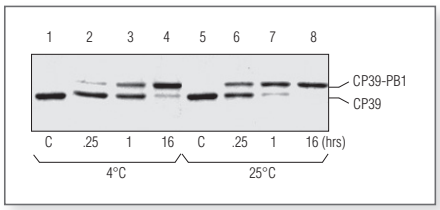
Peptides containing modified residues, such as a phosphorylated tyrosine, can be ligated to a thioester-tagged protein which is generated from an IMPACT C-terminal fusion. The resulting phosphoprotein, containing the carrier protein (CP) joined by a native peptide bond to the phosphopeptide, can be utilized in ELISAs, Western blots or arrays (3-7). The use of ligated carrier protein-peptide substrates enhances sensitivity in ELISAs (Figure 3; 10). In addition, the carrier protein-peptide substrate can be visualized as a single, sharp band on Western blots (Figure 4) and arrays.

Materials

- IMPACT Kit (NEB #E6901)
- Purified protein with a C-terminal thioester - generated using the IMPACT Kit
- Peptide with an N-terminal cysteine; the peptide can contain a fluorescent label, modified amino acid, biotin, etc.
- Abl Protein Tyrosine Kinase, recombinant
- T-Cell Protein Tyrosine Kinase, recombinant

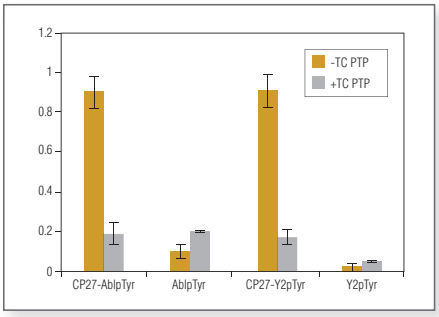
(see other side)

Figure 2: Analysis of peptide ligation by Coomassie Blue stained SDS-PAGE



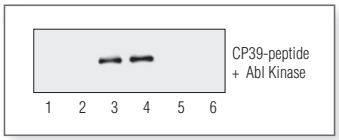
A Control Peptide, PB1, containing an N-terminal cysteine was ligated to a thioester tagged Carrier Protein CP39 (6). The ligation samples were electrophoresed on a 12% SDS polyacrylamide gel and examined by Coomassie Blue staining. Lanes 1 and 5 are controls (C) which contain only Carrier Protein 39.

Figure 3: Enzyme linked immunosorbent assay (ELISA) of protein tyrosine phosphatase (PTP) activity



Phosphotyrosine peptides, AblpTyr and Y2pTyr were ligated to the thioester tagged carrier protein CP27 (3). The ligated phosphoproteins (CP27-AblpTyr and CP27-Y2pTyr) and unligated free peptides (AblpTyr and Y2pTyr) were applied to a 96-well microtiter plate and treated with T-cell protein tyrosine phosphatase (TC PTP, NEB #P0752). Dephosphorylation was detected with a phosphotyrosine antibody (Millipore). Each data point represents the average of three experiments (7).

Figure 4: Western blot analysis of kinase activity



A peptide (Ablpep) containing a candidate phosphorylation site was synthesized with an additional N-terminal cysteine and ligated to CP39. The ligated product was treated with Abl Protein Tyrosine Kinase (NEB #P6050) and then subjected to Western blot analysis using a phosphotyrosine antibody (Cell Signaling Technology). A positive signal was detected only in lanes 3 and 4 where the ligated product was treated with Abl Kinase (6). Lane 1: CP39. Lane 2: CP39 + Abl Kinase. Lane 3: CP39-Ablpep + Abl Kinase (50 units). Lane 4: CP39 Ablpep + Abl Kinase (100 units). Lane 5: CP39-Ablpep. Lane 6: Abl Kinase.

Summary

This application note demonstrates the ability to ligate a protein with a C-terminal thioester that was generating using the IMPACT Kit to a synthetic peptide with an N-terminal cysteine residue. This method of expressed protein ligation can be used for the introduction of modified residues or biotinylated tags.

References

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