

CMC – Structure Initiative Protocols

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Plectin ABD Expression and Purification

The cDNA sequence corresponding to residues 59–293 of human plectin (EMBL accession number U53204) was amplified by polymerase chain reaction (PCR) and cloned into the bacterial expression vector pET15b using NdeI and BamHI restriction sites. The five residue insertion coded by the exon 2 α was introduced by PCR. The absence of errors in the sequences was confirmed by DNA sequencing. Proteins were expressed in *Escherichia coli* strain BL21(DE3). Cell cultures were induced with 0.2 mM isopropylthiogalactoside (IPTG) for 3 hr at 37°C and pellets frozen at –80°C. Upon thawing, cells were sonicated on ice and cell debris removed by centrifugation. The proteins were purified using a nickel-chelating affinity column (Amersham Biosciences, Piscataway, NJ). The His-tag was cleaved by thrombin digestion and removed by extensive dialysis against 20 mM TRIS (pH 7.0), 150 mM NaCl. The purified proteins include four residues (Gly-Ser-His-Met) from the His-Tag at the N terminus.