Two Step Purification of Maltose-binding Protein (MBP) RNase H Fusion Protein with a c-myc Tag and Histidine Tail

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In many cases where the protein is difficult to purify or any specific antibody to the protein is not available, it makes it hard to evaluate the function of the protein. Epitope tagging is a powerful method to characterize a recombinantly expressed protein. Furthermore, recombinant proteins can be purified efficiently using two-step purification system. In this study, we aimed at construction of a vector expressing Maltose-binding protein (MBP) fused RNase H of hepatitis B virus with a c-myc tag and histidine tail at its C-terminal.

Firstly, the c-myc tag and Hisx6 tail on pSecTag vector were amplified with a primer set; Dual-F (5-TTC TGC AGA TAT CCT TAG CAC A-3) and Dual-R (5-GCA AGC TTA AGG CAC AGT CG-3). PCR amplified DNA fragments were cut with *Pst* I and *Hind* III restriction enzymes and cloned into pMAL-c2x digested with the same restriction enzymes. This resultant new vector (pMAL-Dual) has multiple cloning sites (*EcoR* I, *BamH* I, *Xba* I, *Sal* I, *Pst* I, *Not* I, and *Apa* I). RNase H domain was PCR amplified with a primer set; RN-F (5-CCG GGA ATT CCA ACG GCC TGG TCT GTG C-3) and RN-DB (5-AAG GGG CCC CGG TGG TCT CCA TGC-3) excluding a stop codon for cloning into pMAL-Dual. PCR amplified RNase H domain was digested with *BamH* I and *Apa* I restriction enzymes and cloned into pMAL-Dual predigested with the same enzymes (pD-RN). For over expression and purification of pD-RN, induction of culture with 0.2 mM ITPG was performed in 6 hours and purified with amylose affinity chromatography followed by nickel nitriloacetate (NTA) affinity chromatography. Purity of purified pD-RN protein was analysed with SDS-PAGE and Western Blot using rabbit anti-MBP antiserum and mouse anti c-myc monoclonal antibody. After two-step purification step, purity of pD-RN was highly improved.

Two-step dual purification system is a good strategy in recombinant proteins showing difficultly in purifying with one-step purification system.

Key Words: Hepatitis B virus, RNase H, Dual purification system, pMAL-Dual vector