Expression and purification of a subfamily-2 dromedary-derived nanobody by affinity purification of tag-free proteins

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Affinity tags have increasingly facilitated the simple and quick purification of proteins of interest. However, in the majority of methods, the tag removal step remains a challenging issue. Tag removal procedures are inefficient and expensive. The aim of this study was to express and purify nanobody from *E. coli* lysate via split intein mediated ultra-rapid purification (SIRP) technique. SIRP system is based on an engineered variant of a naturally split intein for removing affinity tags and achieving tag-less recombinant proteins. In the version of SIRP employed in this study, chitin-binding domain (CBD) is used as an affinity tag. In this study, we demonstrate how to make the constructs of the camelid nanobody, express the protein in *BL21 (DE3)*, purify it by affinity chromatography (batch and column purification), and confirm the identity of protein with dot blot and western blot techniques. This study shows that using an engineered split intein tag removal system, nanobody is quickly and effectively purified from bacterial lysate.

Key words: Split intein, nanobody, chitin-binding domain, affinity chromatography