



Production of polyclonal antibody against His-tag using recombinant protein containing His-tag

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ABSTRACT

The His-tag is a small epitope tag containing six to nine histidine residues, which is cloned into an expression vector. The vector is then introduced into an expression system such as bacteria. The result is the expression of a recombinant protein carrying a polyhistidine tag at its N- or C-terminus. Anti-His antibodies are suitable for use on western blots, ELISA, immunofluorescence assays, and immunoprecipitations. Anti-histidine antibodies can be used to monitor the progress of purification and the fraction containing the recombinant protein by western blotting, and to verify that no proteolytic degradation of the recombinant protein is taking place. We present here the production of the polyclonal antibody against His-tag. Plasmid pET28a (+) was used to transform *Escherichia coli* BL21(DE3) and express the SUMO protein. The SUMO protein, fused to a 6 His-tag, was purified by affinity chromatography using a Ni-NTA resin. The identity of the purified protein was confirmed by SDS-PAGE. The concentration of *in vitro*-expressed protein was quantified and used for rabbit immunizations. The antiserum was shown to be sensitive and specific for the detection of His-tag in ELISA assays.

Keywords: Anti-His antibody, polyclonal antibody, SUMO protein