ABSTRACT

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Title of diploma thesis: Detection of His-tag and FLAG-tag recombinant proteins

Recombinant proteins are proteins produced in genetically modified organism by recombinant DNA technology that enable insert DNA sequence of target protein in expression cell. This technology allows preparation of actually any protein in any quantity without difficulties associated with demanding purification of native protein from tissues. Recombinant proteins are produced often in form of fusion proteins with suitable peptide tag (e.g. His-tag, FLAG-tag) that can help with their detection and enable simple purification. The aim of this diploma thesis was to introduce and optimize universal method for the detection of recombinant proteins labelled with His-tag (6x histidine) and FLAG-tag. The model recombinant protein with His-tag was enzyme carbonylreductase 1 (CBR1) produced by expression system *Escherichia coli*. For FLAG-tag labelled protein were chosen enzymes DHRS12, HSD11β1, RODH4, DHRS3, DHRS7, DHRS8 produced by insect cells (strain Sf9). SDS polyacrylamide electrophoresis for protein separation and immunochemical detection after Western blotting method with commercially available primary rabbit Anti-6x His tag® - ChlP Grade (abcam®) and secondary polyclonal swine anti-rabbit antibody conjugated with Horseradish peroxidase (Dako) were used. Final vizualization was achieved by chemiluminiscent commercially available kit (Amersham™ ECL™ Prime Western Blotting Detection Reagent, GE Healthcare Life science). Optimization of antibody concentration by dot blot analysis leads to large saving of antibody. This method will be widely used to evaluation of production of diverse recombinant protein at the Laboratory genetically modified organisms, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague.