

Heme ladder, a direct molecular weight marker for immunoblot analysis

Model of one of the used heme ladder proteins, RbsB with a C-terminal heme tag

Invention

The invention provides a novel direct molecular weight marker for immunoblot analysis consisting of heme tagged proteins ranging from 12 kDa to 85 kDa whose enzymatic activities make them detectable simultaneously with the antigen in all peroxidase-based immunoblot systems. The peroxidase activity results from the covalent attachment of heme to selected periplasmic proteins, catalysed by the cytochrome c maturation system of *Escherichia coli* [1]. The newly designed heme-tagged proteins were combined, resulting in the heme ladder, a protein standard suitable for direct molecular weight estimation in immunoblot analysis.

Background

Protein molecular weight markers are widely used in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent immunoblot analysis. In the latter technique, proteins are often detected by enhanced chemiluminescence (ECL) of secondary antibodies that are conjugated to horseradish peroxidase (HRP). However, common protein standards are not detectable by ECL and therefore, no direct molecular weight estimation can be done. Furthermore, directly detectable molecular weight markers for general use in immunoblot analysis require special handling precautions and are available only at high price.

Recently, we have shown that a short heme tag can be used for the covalent attachment of heme to a protein of choice [2]. The resulting heme tagged protein, referred to as artificial c-type cytochrome, was described to exhibit intrinsic peroxidase activity [3].

Advantages

The heme ladder consists of heme tagged proteins that can be detected in an SDS-gel by Coomassie staining and peroxidase activity staining (heme staining) as well as by peroxidase activity staining on a nitrocellulose membrane (ECL). The heme ladder is useful as a novel, direct molecular weight marker in immunoblot analysis as

- (i) there are no additional steps or precautions necessary during immunoblot analysis;
- (ii) it is completely independent of immunological detection by antibodies;
- (iii) costs for materials and working time to produce the heme ladder on a laboratory scale are without optimization 10 to 20-fold lower compared to the price of the protein ladders already available;
- (iv) the marker is stable when kept at 4°C, -20°C and -80°C for up to one year;
- (v) it can be used as a direct marker for proteins in a wide molecular weight range.

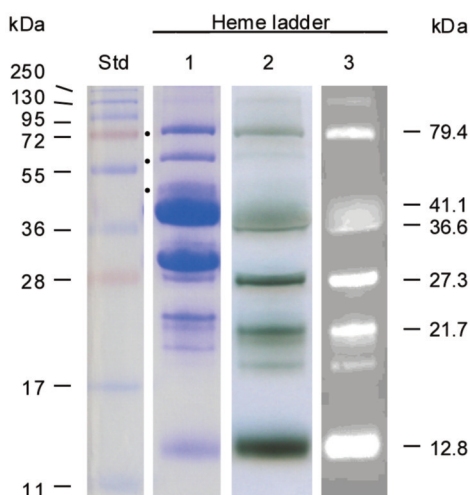


FIG. 1. Molecular weight comparisons of the heme ladder to a commercial protein standard. Proteins were separated by SDS-16% PAGE and stained directly in the gel or immunoblotted and analyzed by enhanced chemiluminescence (ECL). Std, PageRuler™ Plus Prestained Protein Ladder (Fermentas, Lausanne, Switzerland); lane 1, Coomassie stain of the heme ladder; lane 2, heme stain of the heme ladder; lane 3, ECL of the heme ladder. All heme protein dimers are marked by a • in the Coomassie stain.

Applications

Immunoblot analysis is a widely used technique in biochemistry and molecular biology to identify target proteins in a complex protein mixture. The newly developed heme ladder is a molecular weight protein marker that is detectable by a peroxidase activity staining either in a gel or on a nitrocellulose membrane and can thus be used for direct molecular weight estimation of any target protein by ECL detection. Additionally, this marker is visible on an SDS-polyacrylamide gel upon common staining with Coomassie or similar methods.

References

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Keywords

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