

Blue Native Gel Electrophoresis – a valuable tool in studying membrane protein complexes
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Blue-Native Polyacrylamide Gel Electrophoresis (Blue Native PAGE) was originally described by Schägger and von Jagow as a technique for the separation of enzymatically active membrane protein complexes under mild conditions (1). In this variation of gel electrophoresis, the anionic dye Coomassie Brilliant Blue is added to the sample prior to loading and binds to protein complexes during electrophoresis under physiological conditions. The technique has gained interest from researchers focused on functional proteomics in recent years, as it allows the study of protein-protein interactions, and the separation and analysis of very hydrophobic proteins, such as membrane proteins, their complexes, and even super-complexes.

In a further development, Blue-Native PAGE is a fully complementary method to high-resolution two-dimensional electrophoresis and also liquid chromatography of proteins, the most frequently used separation methods in proteomics. The technique is also useful for pre-fractionation of mg amounts of sample for subsequent analysis of smaller protein subsets. In most cases Blue-Native PAGE is combined with a second dimension, which is either a second Blue-Native PAGE after equilibration with a medium-mild detergent, or SDS PAGE for mapping of the related subunits. A comprehensive review of applications of this method has recently been published (2).

Hydrophobic proteins and complexes are first solubilized with a mild nonionic detergent, like Triton X-100 or digitonin. Digitonin, the preferred detergent as it is the mildest, allows the separation of intact super-complexes. Coomassie Blue is added to the sample and cathodal running buffer and remains bound to all hydrophobic proteins and to many water-soluble proteins by hydrophobic interactions even when an electric field is applied. Coomassie Blue is anionic so all protein-dye complexes become negatively charged in the pH 7.5 buffer used, and the complexes migrate towards the anode. Separation of protein complexes occurs according to size in the range 10 kDa to 10 MDa. These protein-dye-complexes are soluble in the absence of detergent, which minimizes the risk of denaturation. Aggregation of the proteins is also prevented because of their overall negative charge. Detection of the proteins and complexes is straightforward as the attached blue dye makes them visible. Porosity gradient gels from 4 to 16% T are employed: this allows large super complexes to enter the gel, prevents small complexes and single proteins from migrating out of the gel, and applies a band-sharpening effect.

After the first dimension electrophoresis is complete, the lanes containing the separated complexes are cut out with a sharp knife or ruler edge, equilibrated in SDS solution, and embedded into a stacking gel layer of a second dimension discontinuous SDS gel. During this process the complexes fall apart into their components (subunits) to form protein-SDS micelles that separate in the SDS gel according their molecular sizes. A Tris-tricine buffer system is preferred over the conventional Tris-glycine gel, because it offers an improved resolution of low molecular weight proteins. Gels containing non-labeled proteins can be stained after the separation with Coomassie Blue, silver stain, or with a fluorescent stains such as Deep Purple or Sypro Ruby.

A more recent development, Blue-Native DIGE, employing Blue-Native PAGE of fluorescent-labeled protein complexes with subsequent SDS PAGE, is perfectly suited for detecting

biologically induced changes in proteomics experiments (3). It has the advantage of eliminating gel-to-gel variation. The CyDyes are added to the complexes prior to the Coomassie Blue dye. The different samples labeled with different fluorophores are combined and applied together in one lane. After the second dimension SDS PAGE, the gels are scanned with a multi-fluorescence imager. Results are shown in Figure 1.

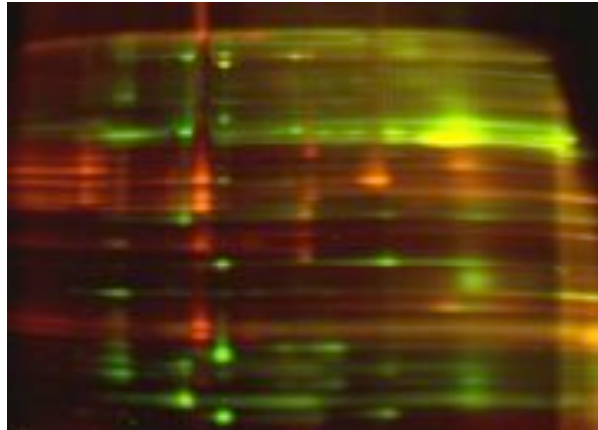


Figure 1: False color display of two fluorescently labeled samples separated with Blue Native DIGE. Complex proteins of Arabidopsis mitochondria (Cy3, green) respiratory system and chloroplasts (Cy5, red) photosynthesis system.

Blue native PAGE gels are interpreted in a different manner than conventional 2-D gels. Vertically aligned spots indicate the protein composition of a protein complex. Larger complexes and super complexes are located on the left hand side of the image. Blue Native DIGE is a useful additional method for studying membrane proteins and protein complexes and offers some distinct advantages over conventional 2-D electrophoresis or liquid chromatography techniques.

References

1. Schägger, H. and Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes. *Anal Biochem.* **199**, 223-231.
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3. Perales, M. et al. (2005) Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces complex I and supercomplex. *J Mol Biol.* **350**, 263-277.
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