**Biology and Biochemistry Department**

**BIOC 312**

**Biochemistry II lab**

**Experiment #9**

**Sec 1**

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**Title**

**Separation of Mitochondrial Electron Transport Chain Complexes by Blue Native PAGE2**

* **INTRODUCTION:**

There are 3 types of Polyacrylamide gel electrophoresis that we will discuss in this article and these PAGES are Clear-native (CN-PAGE) then Blue native (BN-PAGE) and finally sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

CN-PAGE First we have which have several Limitations and advantages, such that the CN PAGE in an acrylamide gradient gel, he's act separates acidic water-soluble and membrane proteins (pI < 7) and has lesser resolution than blue-native PAGE (BN-PAGE). Colorless native PAGE was the original name for this type of PAGE.1

So when compared to BN-PAGE, which employs negatively charged protein-bound Coomassie-dye to force a charge shift on the proteins, this complicates estimate of native masses and oligomerization states. As a result, for standard analyses, BN-PAGE rather than CN-PAGE is frequently used and commonly. 1

Anyway, there are many advantages to CN-PAGE including: identification of catalytic activities, and membrane protein complexes may be separated at a microscale with ease. An example is the determination of the catalytic activities in CN-PAGE mitochondrial ATP synthase such that Sensitivity was at least 10-times higher compared to the in gel assay in BN-gels. The in-gel ATP hydrolysis assay for yeast mitochondrial ATP synthase may be made much more sensitive using CN-PAGE. Unlike BN-PAGE, its activity was sensitive to an ATP synthase inhibitor.An example of microscopic separation is fluorescence resonance energy transfer (FRET) analyses.1

However, the CN-PAGE is a milder version of BN-PAGE. Due to digitonin and CN-PAGE, in particular, can preserve labile supramolecular assemblies of membrane protein complexes that are dissociated under BN-PAGE conditions. CN-PAGE was used to identify enzymatically active oligomeric forms of mitochondrial ATP synthase that were previously undetectable using BN-PAGE. 1 2

Also the CN-PAGE could not be improved to achieve the high-resolution power of BN-PAGE, the milder circumstances of CN-PAGE appeared to be a potential advantage, due to allowing for the identification of additional physiological protein assemblies previously undetected by BN-PAGE.3 5 The native complexes and supercomplexes were separated using one-dimensional CN-PAGE. The complexes in supercomplexes were ideally detected using 2-D BN-PAGE, while the protein subunits may be identified using 3-D SDS-PAGE as an option.3

Then the separation and proteomics analysis of high-molecular-weight protein complexes using blue native polyacrylamide gel electrophoresis (BN–PAGE) is a strong approach. It's frequently done using gradient gels and is commonly used to investigate mitochondrial membrane complexes involved in electron transport and oxidative phosphorylation.4

The Blue native PAGE (BN-PAGE) has several advantages, including the ability to isolate protein complexes from biological membranes and entire cell and tissue homogenates in a one step. It may also be utilized to discover physiological protein–protein interactions and determine native protein masses and oligomeric states. Electroelution or diffusion are employed to extract native complexes from gels, which are then utilized for 2D crystallization and electron microscopy, or evaluated using in-gel activity assay or native electroblotting and immunodetection.5 The BN-PAGE was developed to separate mitochondrial membrane proteins and complexes with masses ranging from 10 kDa to 10 MDa.6 7 Also the BN-PAGE is a simple and inexpensive technique based on a few basic, easy principles.

Biological membranes are solubilized by nonionic detergents. And the stability of the protein complexes of interest determines the nonionic detergent to use. Mildest detergent which is digitonin can be used or Dodecylmaltoside which is mild neutral detergent can be used. The digitonin is thus used to determine physiological protein–protein interactions without the use of chemical crosslinking by isolating supramolecular associations of multiprotein complexes. While dodecylmaltoside although it is useful for isolating membrane proteins and individual complexes, it commonly dissociates labile hydrophobic interactions.5

SDS-PAGE is the type of polyacrylamide gel electrophoresis. Ulrich K. Laemmli developed a discontinuous electrophoretic system that is commonly used to separate proteins with molecular masses between 5 and 250 kDa. The combination of sodium dodecyl sulfate and polyacrylamide gels together eliminates the effects of structure and charge, allowing proteins to be separated only entirely on the basis of molecular weight differences.8

Also, a gram of protein binds around 1.4 grams of SDS, or one SDS molecule for every two amino acids. SDS acts as a surfactant, obscuring the intrinsic charge of the proteins and resulting in charge-to-mass ratios that are remarkably comparable. The intrinsic charges of the proteins are negligible in comparison to the SDS loading, and the positive charges are also greatly reduced in the basic pH range of a separating gel. When a steady electric field is applied, the proteins migrate towards the anode at varying speeds depending on their mass. This straightforward method allows for exact protein separation based on mass.9

Small proteins pass through the mesh of the gel relatively quickly, but larger proteins are more likely to be retained and thereby more slowly, allowing proteins to be separated by molecular size. Depending on the voltage and length of gel employed, electrophoresis might last anywhere from half an hour to several hours.9

The discontinuous SDS-PAGE method is the most widely utilized. In this approach, the proteins migrate first into a neutral pH collecting gel, where they are concentrated, and then into a basic pH separating gel, where the real separation takes place. Stacking and separating gels differ by different pore size (4-6 % T and 10-20 % T), ionic strength and pH values (pH 6.8 or pH 8.8).9

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