Native PAGE

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Native-PAGE

- The original discontinuous gel system was developed by Ornstein and Davis (1964) for the separation of serum proteins in a manner that preserved native protein conformation, subunit interactons, and bioloical activity.
- In such systems, proteins are prepared in nonreducing, nondenaturing sample buffer, and electrophoresis is also performed in the absence of denaturing and reducing agents.
- Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used to obtain high resolution separation of complex mixtures of proteins.
- The method initially denatures the proteins that will undergo electrophoresis.
- Although covalent structural features of resolved proteins can be determined with SDS-PAGE, functional properties are destroyed, including the presence of non-covalently bound metal ions.

Native-PAGE

- Native PAGE is one of the most powerful techniques for studying the composition and structure of native proteins, since both the conformation and biological activity of proteins remain intact during this technique.
- Often, it is difficult to find standard proteins that resemble the shape, partial specific volume and degree of hydration as the native protein under investigation .
- Gradient gels for native PAGE sharpen the protein bands and allow complex mixtures of proteins to be separated on a single gel.
- There is no universal gel chemistry system ideal for the electrophoresis of all proteins in their native state.
- Protein stability, resolution and isoelectric point are important considerations for the buffer selection.

Native PAGE Principle

- In native PAGE, proteins are separated according to the net charge, size, and shape of their native structure.
- Electrophoretic migration occurs because most proteins carry a net negative charge in alkaline running buffers.
- The higher the negative charge density (more charges per molecule mass), the faster a protein will tend to migrate.
- At the same time, the frictional force of the gel matrix creates a sieving effect, regulating the movement of proteins according to their size and three-dimensional shape.
- Small proteins face only a small frictional force, while larger proteins face a larger frictional force.
- Thus, native PAGE separates proteins based upon both their charge, mass and structure.
- Because no denaturants are used in native PAGE, subunit interactions within a multimeric protein are generally retained and information can be gained about the quaternary structure.
- In addition, some proteins retain their enzymatic activity (function) following separation by native PAGE.
- Thus, this technique may be used for preparation of purified, active proteins.

Of all the available methods in native fractionation, Blue Native PAGE (BN-PAGE) and Colorless Native PAGE (CN-PAGE) are most preferred

- Based on the success of denaturing PAGE, native PAGE systems have been explored and developed.
- One of the most remarkable developments is the so-called blue native (BN-) PAGE and its further development clear native (CN-) PAGE.
- These methods use non-ionic detergents with or without the addition of Coomassie in combination with the near neutral buffer capacity of Bis-TRIS (bis(2-hydroxyethyl)-amino-tris(hydroxymethyl)-methane).

Blue native polyacrylamide gel electrophoresis

- Blue native polyacrylamide gel electrophoresis is a very common method implicated for characterization of proteins in their enzymatically active state with a high-resolution separation.
- In this method, a dye, Coomassie Blue-G250, is used for visualization and induction of external negative charge on the protein complexes.
- The complexes are then separated based on their molecular weights contrary to that of conventional SDS-PAGE in which the proteins are fractionated based on their charge/mass ratio.
- In BN-PAGE, the protein complexes tend to migrate according to the pore size of the gradient gel till they reach the pore size limit point.
- Coomassie Blue-G250 dye provides many advantages over SDS such as:
  - It is anionic in nature; hence allow coating of the complexes with negative charge without denaturation.
  - The solubility is higher in water
  - It has a unique capability of binding with membrane proteins, therefore the best tool for membrane proteins fractionation. There is a minimal probability of aggregation of membrane proteins because of the charge distribution.
  - Also, the interaction of the dye with the membrane proteins facilitates the loss of their hydrophobicity, eventually making them water-soluble.

BN-PAGE

- BN-PAGE has a very high resolution ranging from 100 kDa to 10 MDa and it is largely dependent on the concentration range of acrylamide and quality of the gradient gel.
- However, other things are important for better results such as low concentration of mild detergents such as digitonin or dodecylmaltoside and the type of sample for extracting proteins.
- BN-PAGE uses gradient polyacrylamide gel for separation.
- Coomassie blue G-250 is added to the lysate obtained after the solubilization and centrifugation of the sample.
- During the process of separation, the native proteins and complexes migrate as blue dots or bands through the gradient gel.
- **Advantages of BN-PAGE:**
  - Transient interactions can also be studied as the conditions are non-denaturing.
  - Two-dimensional BN-PAGE provides subunit composition information.
  - It is an exquisite tool for studying comparative protein associations after experimental conditions
  - BN-PAGE confirms the results of immunoprecipitation.

BN-PAGE

- **Limitations of BN-PAGE:**
- It requires good quality and robust antibodies for detection of the protein. Not all antibodies that are helpful in SDS-PAGE can provide satisfying results with BN-PAGE.
- The resolution is low between protein complexes which require optimization of the gradient gel every time.
- Coomassie dye has shown to interact with few protein-protein complexes resulting in a smear formation on the gel. In such cases, CN-PAGE is preferred over BN-PAGE.

CN-PAGE

- Clear-native PAGE (CN-PAGE) separates acidic water-soluble and membrane proteins (pI < 7) in an acrylamide gradient gel, and usually has lower resolution than blue-native PAGE (BN-PAGE).
- The migration distance depends on the protein intrinsic charge, and on the pore size of the gradient gel.
- This complicates estimation of native masses and oligomerization states when compared to BN-PAGE, which uses negatively charged protein-bound Coomassie-dye to impose a charge shift on the proteins.
- Therefore, BN-PAGE rather than CN-PAGE is commonly used for standard analyses.
- However, CN-PAGE offers advantages whenever Coomassie-dye interferes with techniques required to further analyze the native complexes, e.g., determination of catalytic activities, as shown here for mitochondrial ATP synthase, or efficient microscale separation of membrane protein complexes for fluorescence resonance energy transfer (FRET) analyses.
- CN-PAGE is milder than BN-PAGE.
- Especially the combination of digitonin and CN-PAGE can retain labile supramolecular assemblies of membrane protein complexes that are dissociated under the conditions of BN-PAGE.
- Enzymatically active oligomeric states of mitochondrial ATP synthase previously not detected using BN-PAGE were identified by CN-PAGE.