MODIFIED BLUE NATIVE ELECTROPHORESIS PROTOCOL FOR THE SEPARATION OF MITOCHONDRIA PROTEIN COMPLEXES

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The major part of the chemical energy produced inside the cell results from ATP-oxidative phosphorylation in mitochondria. Many mitochondria proteins are organized into complexes, located in its inner membrane. Blue Native electrophoresis (BN-PAGE) allows the isolation of those proteins in their native form. In this study we have employed a straightforward BN-PAGE protocol for the preparation of native gels of inner mitochondria membrane samples (20µg of proteins) from red and white gastrocnemius muscle of Wistar rats. Running and stacking gels (7,5% and 3%) were used; the running buffer contained 250mM Tris and 1.9M glycine. Electrophoresis was carried out at ambient temperature, under 100V until the blue dye reached the end of the gel. We have introduced modifications in the protocol described in the literature (Schägger & von Jagow, Anal. Biochem. 199:223, 1991) getting to a simpler protocol with equivalent protein resolution and low costs. The novel protocol presented has an additional advantage, since the preparation gel and reagents are the same required for subsequent 2D electrophoresis. From the original BN-PAGE protocol G-250 Comassie Blue, Bis-Tris, EDTA and aminocaproic acid were the preserved reagents, but only in the sample preparation buffer and not in the gel and running buffers. The use of different detergents in this protocol is now under investigation.
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