Subunit stoichiometry determination by perfluorooctanoic acid polyacrylamide gel electrophoresis (PFO-PAGE)

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Method Article

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Abstract

Introduction

This procedure describes how to assess the native quaternary structure of membrane proteins by PAGE using mild solubilization in the non-dissociative detergent perfluoro-octanoic acid \(PFO). The number of subunits is deduced from the molecular mass of the preserved homo-multimeric protein complex. The PFO-PAGE technique was first described by Ramjeesingh, M _et al._ \(1999) and applied later to determine the subunit stoichiometry of other channels \(_e.g._ Kedei, N. _et al._ \(2001) and in the present study to Orai and P2X2 expressed in _Drosophila_ S2 cells or human embryonic kidney HEK293 cells).

Procedure

1- Rinse the cells twice with ice-cold phosphate-buffered saline \(PBS). 2- Harvest the cell pellet in ice-cold PBS supplemented with protease inhibitors and sonicate. Alternatively, solubilize the cells in the presence of 1% NP-40, a mild detergent diluted in PBS, for 20 min at 4°C under agitation and centrifuged at 16,000 × _g_ for 10 min to remove cellular debris. 3- Measure the protein content by a small-volume micromethod using the Bio-Rad D_C protein assay. 4- Mix 30-40 µg of lysates at 2 µg/µl of protein with doubly concentrated PFO sample buffer \(100 mM Tris base, 2-8% NaPFO \(Oakwood Products Inc), 20% glycerol, and 0.005% bromophenol blue, pH adjusted at 8.0 with NaOH) and 25 mM DTT. 5- Incubate at room temperature for 25 min, vortex briefly, and centrifuge 5 min at 10,000 × _g_. 6- Load your samples at room temperature on a 4-12% precast gradient Novex Tris-Glycine gel without SDS \(Invitrogen) along with molecular weight standards resuspended in PFO sample buffer \(high-molecular-mass rainbow marker kit \(GE Healthcare), cross-linked Albumin and phosphorylase b \(Sigma)). 7- Perform electrophoresis at 140 V with a running buffer \(25 mM Tris, 192 mM glycine, 0.5% NaPFO, pH 8.5, adjusted with NaOH) precooled to 4°C. During the run, the electrophoresis box is kept at 4°C. 8- Transfer the proteins to a nitrocellulose membrane for 1 hr in a transfer buffer containing 25 mM Tris, 192 mM glycine and 20% \(v/v) methanol. 9- Analyze the protein band sizes either by Amido black staining \(Sigma) for the molecular weight standards or by standard immunoblotting.

References

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