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

CURRENT STATUS: POSTED

Aubin Penna
Department of Physiology and Biophysics, University of California, Irvine, California, USA 92697
apenna@uci.edu*Corresponding Author*

DOI:
10.1038/nprot.2008.210

SUBJECT AREAS
Biochemistry *Computational biology and bioinformatics*

KEYWORDS
stoichiometry, perfluorooctanoic acid, non-dissociative electrophoresis, quaternary structure

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

Ramjeesingh, M., Huan, L. J., Garami, E. & Bear, C. E. Novel method for evaluation of the oligomeric structure of membrane proteins. *Biochem. J.* **342** (Pt 1), 119-23 (1999).

Kedei, N. *et al*. Analysis of the native quaternary structure of vanilloid receptor 1. *J. Biol. Chem.* **276**, 28613-9 (2001).

The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers

by Aubin Penna, Angelo Demuro, Andriy V. Yeromin, +3

Nature (08 September, 2008)