Protein gel blot analysis

Dominique Loqué
Carnegie Institution
Wolf Frommer
Carnegie Institution

Method Article

Keywords: yeast, Western, protein

Posted Date: March 8th, 2007

DOI: https://doi.org/10.1038/nprot.2007.126

Abstract

Introduction

Characterization of protein levels using a specific antiserum.

Procedure

1) Grow yeast cells carrying the empty vector, pDR-AMT1;1, pDRAMT1;1-T460A or pDR-AMT1;1-T460D plasmids, in liquid YNB media supplemented with 3% glucose and 5mM arginine. 2) Harvest cells at OD_{600} 0.6-0.8 and chill on ice. 3) Harvest cells from 100 mL culture by centrifugation at 5,000g for 10 min at 4°C and wash once with half volume of chilled water. 4) Resuspend cells in 200 µL of chilled extraction buffer \(20 mM Tris-HCl, 10 mM MgCl2, 1 mM EDTA, 5% Glycerol, 1 mM DTT, 1 mM PMSF and 1x protease inhibitor cocktail \(Sigma-Aldrich, St. Louis, Mo), pH 8) and disrupt with glass beads. 5) Lyze cells with 10 vortex cycles \(30 sec each) alternating with ice incubation. 6) Collect supernatants after centrifugation at 12,000g for 5 min. 7) Resuspend cell debris and glass beads in 1mL of extraction buffer, vortex twice for 30 sec and centrifuge again. 8) Collect the supernatant and pool with the first one. 9) Harvest the microsomal fraction by centrifugation at 100,000g for 50 min at 4°C. 10) Resuspend the pellet in 200 µL of storage buffer \(20 mM Tris-Cl, 0.1 mM EDTA, 10% glycerol, 100 mM KCl, 1 mM DTT, 1 mM PMSF and 1x protease inhibitor cocktail, pH7.5). 11) Estimate protein concentration by Bradford protein assay \(Bio-Rad, Hercules, CA) using BSA as standard. 12) Denature proteins \(5 µg per lane) in loading buffer \(62.5 mM Tris-HCl pH 6.8, 10% v/v glycerol, 2% w/v SDS, 2.5% v/v β-mercaptoethanol, 0.01% w/v bromophenol blue) at 37°C for 30 min, separate on 10% SDS-PAGE gels and transfer to PVDF membrane. 13) We raised a polyclonal antibody against a peptide corresponding to the Cterminus of AMT1;1 \(n-RRVEPRSPSPSGANT-c) \(Biotrend, Köln, Germany). The antiserum was affinity-purified as described in ref 1. Dilute the primary antibody \(1/1,000) and secondary antibody \(1/10,000; peroxidase-conjugated anti-rabbit IgG - Pierce Biotechnology, Rockford, IL) in blocking solution. Use rainbow marker \(Amersham Biosciences, Piscataway, NJ) as a molecular mass marker. Develop blots using SuperSignal West Pico Chemiluminescent Substrate \(Pierce Biotechnology, Rockford, IL).

References

\(1) Ludewig, U. _et al._ Homo- and hetero-oligomerization of ammonium transporter-1 NH_4^+ uniporters. _J. Biol. Chem._ **278**, 45603-45610 \(2003).