QPNC-PAGE Standardized Protocol

For Acidic, Basic and Neutral Metalloproteins

MW 6 - > 200kDa

USE ONLY FRESH GELS

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Recommended Tools (Bio-Rad)

Model 491 Prep Cell (U.S. patent no. 4,877,510)
Power Pac 1000: Constant Power: 5 W; Time: 8 hr
Model EP-1 Econo Pump: 1 mL/min; 5 mL/fraction; 80 mL prerun V; 480 ml total V (Eluent)
Model 2110 Fraction Collector: 80 Fractions
Model EM-1 Econo UV Monitor: AUFS 1.0; Detection Wavelength 254 nm
Model 1327 Econo Recorder: Range: 100 mV; Chart Speed: 6 cm/hr
Model SV-3 Diverter Ventil
Buffer Recirculation Pump: Flow Rate: 95 mL/min (Electrophoresis Buffer)

Stock Solutions

1) 200 mM Tris-HCl 10 mM NaN_3 pH 10.00 – Keep RT.
2) 200 mM Tris-HCl 10 mM NaN_3 pH 8.00 – Keep RT.
3) 40 % Acrylamide/Bis 2.67 % C - Keep 4°C.
4) 10 % Ammonium Persulfate (APS) - Keep 4°C (freshly prepared).

Electrophoresis Buffer

20 mM Tris-HCL 1 mM NaN_3 pH 10.00 degassed – Keep 4°C

Upper Electrophoresis Chamber (Prep Cell): 500 mL Electrophoresis Buffer
Lower Electrophoresis Chamber (Prep Cell): 2000 mL Electrophoresis Buffer

Eluent

20 mM Tris-HCL 1 mM NaN_3 pH 8.00 degassed – Keep 4°C

Elution Chamber (Prep Cell): 700 mL Elution Buffer

Separating Gel

Acrylamide 4% T Volume: 40 mL

ingredients:

4 mL 40 % Acrylamide/Bis 2.67 % C

4 mL 200 mM Tris-HCl 10 mM NaN_3 pH 10.00
32 mL H_2O
200 µL 10% APS
20 µL TEMED

Add TEMED and APS at the end. Gently swirl the flask to mix, being careful not to generate bubbles. Pipette the solution to a level of 40 mm in a graduated glass column with an inner diameter of 28 mm. Add 3 mL of 2-propanol. After 60 minutes of polymerization rinse the surface of the gel with electrophoresis buffer and then cover the gel surface with 4 mL electrophoresis buffer. ***The total time of polymerization is 69 hr at RT***. The emerging heat during the polymerization processes is dissipated according to the manufacturer's manual.

Sample Preparation and Separation

Keep samples (biofluids) at 4°C. Volumes of 0.3 mL Glycerol and 2.7 mL sample aliquot are mixed 5 minutes before the electrophoretic separation is started. Then the prepared sample is carefully layered under the upper electrophoresis buffer on the gel surface and separated in ring-shaped protein bands. The proteins are charged either negatively (pI<10.0) or positively (pI >10.0) under the conditions of the electrophoresis buffer and migrate from the cathode to the anode or from the anode to the cathode in the electrical field. The separated protein molecules are continuously eluted by a physiological eluent in a special elution chamber and transported to a fraction collector. The separation system is cooled in a refrigerator at 4°C.

Purified, partially purified and raw samples may be used. ***Sample preparation steps like protein precipitation should be avoided because of the danger of protein denaturation***. The chemical stability of the separated metalloproteins can be investigated and confirmed by using preparative native gel permeation chromatography.

Application and Quantitation

Quantitative **P**reparative **N**ative **C**ontinuous **P**oly**a**crylamide **G**el **E**lectrophoresis (QPNC-PAGE) is a very efficient technique to isolate native or active metalloproteins in biological samples and to resolve properly and improperly folded metal cofactor-containing proteins in complex protein mixtures.

Fe, Cu, Zn, Ni, Mo, Pd, Co, Mn, Pt, Cr, Cd and other metal cofactors can be identified and quantified by ICP-MS (Abbr.: **i**nductively **c**oupled **p**lasma **m**ass **s**pectrometry), for example. Because of high purity and optimized concentration of the different metalloproteins (e.g., Cu chaperone for superoxide dismutase, prions and metalloenzymes) in specific PAGE fractions, the related structures of these analytes can be elucidated by using solution NMR spectroscopy under non-denaturing conditions.

Reference

International Patent Application Number: PCT/DE2004/001514.