

QPNC-PAGE: Standard Protocol

For Acidic, Basic and Neutral Metalloproteins

MW 6 - \geq 200 kDa

USE ONLY FRESH GELS

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Abstract: Quantitative Preparative Native Continuous Polyacrylamide Gel Electrophoresis (QPNC-PAGE) is a high-resolution method to isolate quantitative amounts of native or active metalloproteins in biological samples and to resolve properly- and improperly-folded metal cofactor-containing proteins or protein isoforms in complex protein mixtures.

METHOD

Table1. Recommended Equipment and Run Conditions

Model 491 Prep Cell (<i>Fig. 1</i>)
Power Pac 1000: Constant Power: 5 W; Time: 480 min
Model EP-1 Econo Pump: 1 mL/min; 5 mL/Fraction; 80 mL Prerun Volume; 480 mL Total Volume (Elution Buffer)
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)

All instruments are from Bio-Rad Laboratories, Inc.

SOLUTIONS

-Stock Solutions

200 mM Tris-HCl 10 mM NaN₃ pH 10.00 – Store at room temperature

200 mM Tris-HCl 10 mM NaN₃ pH 8.00 – Store at room temperature

40 % Acrylamide/Bis 37.5 : 1 - Store at 4°C

10 % Ammonium Persulfate (APS) - Store at 4°C (freshly prepared)

-Electrophoresis Buffer

20 mM Tris-HCl 1 mM NaN₃ pH 10.00 – Store at 4°C and degas before use

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 NaN₃ pH 8.00 – Store at 4°C and degas before use

Elution Chamber (Prep Cell): 700 mL Elution Buffer

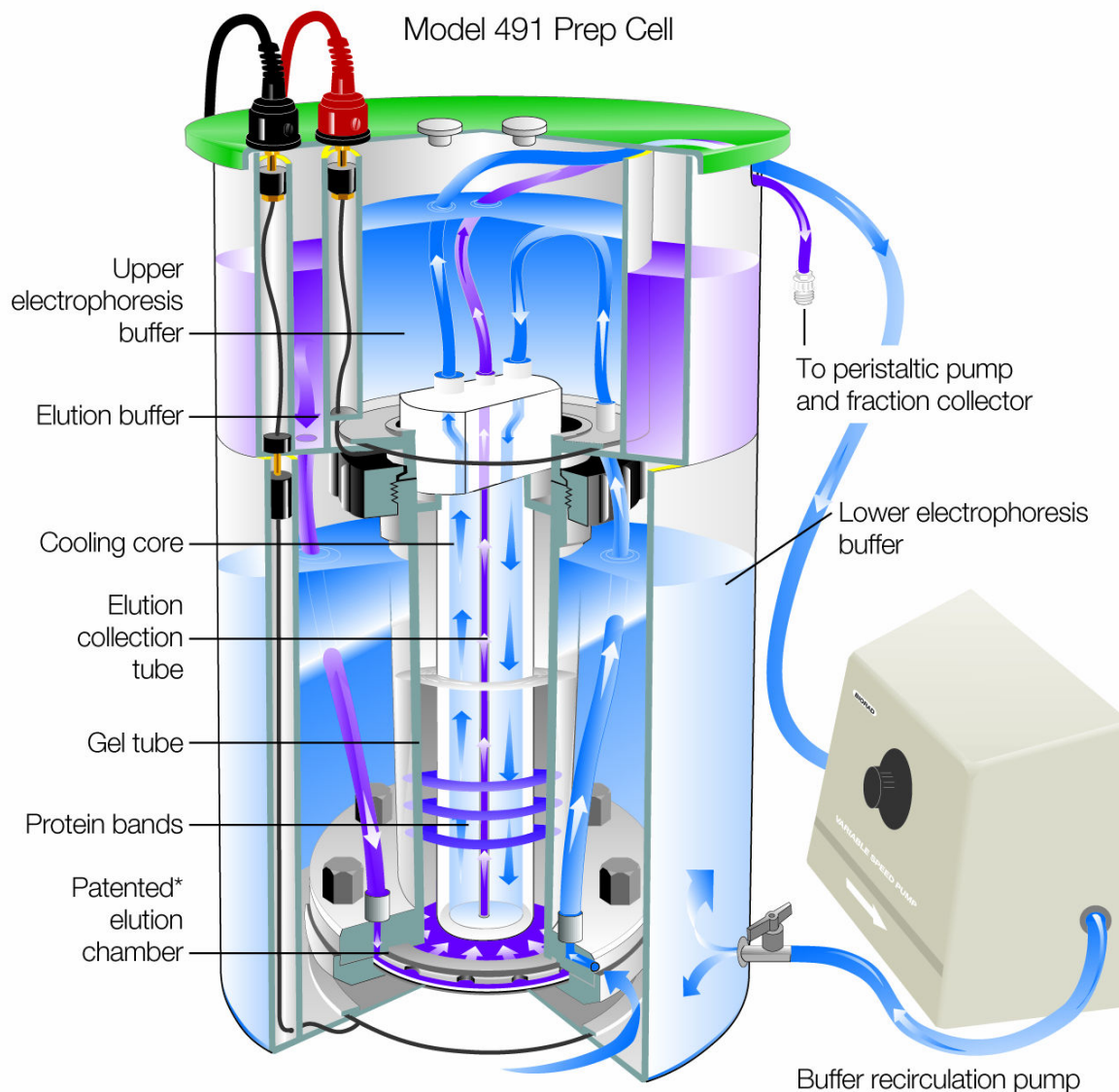


Figure 1: Electrophoresis chamber “Model 491 Prep Cell” for separating quantitative amounts of metal cofactor-containing proteins in complex protein mixtures. This figure is used with the permission of Bio-Rad Laboratories, Inc..

GEL

-Separating Gel

Acrylamide: 4%T - 2.67%C

Ingredients (40 mL gel):

4 mL 40 % Acrylamide/Bis 37.5:1

4 mL 200 mM Tris-HCl 10 mM NaN₃ pH 10.00

32 mL H₂O

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 (*Model 491 Prep Cell* equipment). Add 3 mL of 2-propanol. After 60 minutes of polymerization time rinse the surface of the gel with electrophoresis buffer and then cover the gel surface with 4 mL electrophoresis buffer. [Allow the polymerization reaction to proceed for 69 hours at RT.](#) The heat generated during the polymerization processes is dissipated according to the *Model 491 Prep Cell* instruction manual. There is no stacking gel.

PROTEIN SEPARATION

Keep liquid samples at 4°C. Gently mix 0.3 mL of glycerol and 2.7 mL of sample containing < 0.5 mg protein. Begin a prerun at 5 W constant power (*Tab. 1*). After 75 minutes interrupt the power for 5 minutes and underlay the sample mixture below the upper electrophoresis buffer on to the gel surface (*Fig. 1*). This gives ample time in which to gently load the sample and for the sample solution to settle on to the top of the PAGE gel. After that begin the electrophoretic separation at 5 W constant power. The protein mixture is separated in ring-shaped protein bands (*Fig. 1*). The separated protein molecules are continuously eluted by the *Prep Cell* into the physiological eluent and transported to a fraction collector (*Fig. 1*). [The equipment \(except Recorder and Power Pac\) is cooled in a refrigerator at 4°C \(Tab. 1\).](#) Protein precipitation or other sample concentration steps should be avoided at all times of the analytical process to avoid the possibility of protein denaturation or aggregation.

QUANTITATION OF METAL COFACTORS

As an example, Fe, Cu, Zn, Ni, Mo, Pd, Co, Mn, Pt, Cr, Cd and other metal cofactors can be identified and quantified in physiological concentrations by [ICP-MS](#) (Abbr.: [inductively coupled plasma mass spectrometry](#)). Because of high purity and optimized concentration of native and denaturing metalloproteins (e.g., Cu chaperone for superoxide dismutase, prions or metalloenzymes) in specific PAGE fractions, the related structures of these analytes can be elucidated by using solution [NMR](#) (Abbr.: [nuclear magnetic resonance](#)) spectroscopy under non-denaturing conditions. Thus, the QPNC-PAGE procedure may contribute to the diagnosis and therapy of diseases concerning biometal metabolisms (e.g., Cu, Zn, Fe) and help to develop metal-based medications for the treatment of organisms with protein-misfolding diseases.

REFERENCE

Bernd Kastenholz "METHOD FOR ISOLATING METAL COFACTORS OUT FROM BIOLOGICAL-ORGANIC SYSTEMS INVOLVING THE USE OF PREPARATIVE NATIVE CONTINUOUS POLYACRYLAMIDE GEL ELECTROPHORESIS (PNC-PAGE)" International Patent Application Number: PCT/DE2004/001514; Publication Number: WO/2005/014147; Publication Date: 17 February 2005.

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