

Supplementary Material

Kinetic Features of 3'-5' Exonuclease Activity of Human AP-Endonuclease APE1

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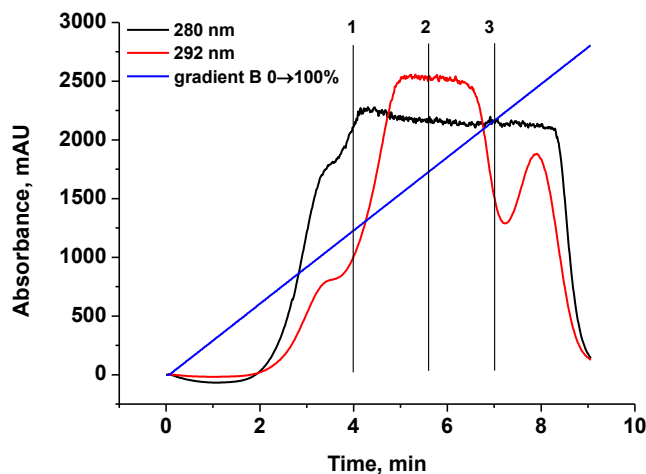
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Protein purification

The cell lysate was centrifuged (40,000×g, 40 min), and the supernatant was loaded onto column I (Q-Sepharose Fast Flow, Amersham Biosciences, Sweden) with subsequent washing with buffer solution I (20 mM HEPES-NaOH, pH 7.8) containing 100 mM NaCl. Fractions containing the protein were collected and loaded onto column II (HiTrap-Heparin™ Amersham Biosciences, Sweden) in buffer solution II (20 mM HEPES-NaOH, pH 7.8) containing 40 mM NaCl. Chromatography was run in buffer solution II and a linear gradient of 40 → 600 mM NaCl. The solution's absorbance was detected at a wavelength of 280 nm and 292 nm (Figure S1A). The protein purity was determined by gel electrophoresis (Figure S1B). Fraction 2 (see lane 7) was used in the work.

A



B

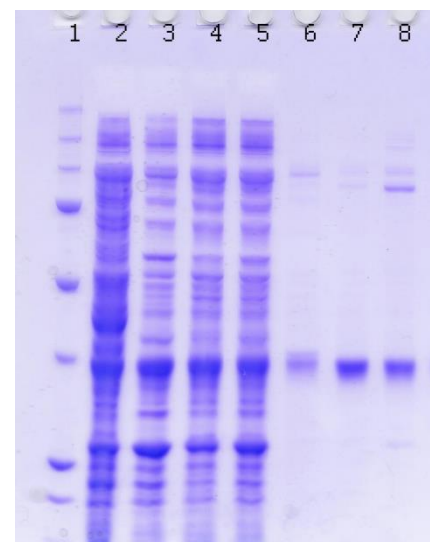


Figure S1. APE1 purification. (A) Proteins elution from Heparin column in a 40–600 mM NaCl gradient. (B) PAGE analysis of APE1 purification: 1, protein marker (250, 150,100, 75, 50, 37, 25, 20 kDa); 2, total cell lysate; 3, flow through Q sepharose (last drop); 4, flow through Q sepharose (for loading on Heparin); 5, flow through Heparin; 6, fraction 1; 7, fraction 2; 8, fraction 3.