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Single-step purification by heat shock

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ABSTRACT

After the cultivation period, cells were collected by centrifugation at 3500 × g for 30 min at 4 °C and resuspended in 4 ml lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM Imidazole and 0.05% Tween 20 at a pH of 7). The mixture was sonicated with 4 × 40 sec pulses followed by 20 sec rest between cycles at 4 °C. The crude extracts were then centrifuged at 9000 × g for 30 min at 4 °C, and the resulting supernatant was heat-treated in a hot water bath at 90 °C for 15 min. Insoluble material was separated by centrifugation at 13000 × g for 10 min, and the supernatant, containing the enzyme, was analyzed on a 12% SDS–polyacrylamide. The purified elution fractions were dialysed overnight in a 50 mM potassium-phosphate buffer (pH=7) at 4 °C.

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- 1 Cultivation cells 50 mL 37 °C Overnight , collected by centrifugation 3500 x g, 4°C, 00:30:00 and resuspended in 4 ml lysis buffer (50 millimolar (mM) NaH₂PO₄ , 300 millimolar (mM) NaCl , 10 millimolar (mM) Imidazole and 0.05 % volume Tween 20 at 7)
- 2 The mixture was sonicated with 4 × pulses 00:00:40 Amper 0.7 followed by 00:00:20 rest between cycles at 4 °C .
- 3 The crude extracts were then centrifuged 9000 x g, 4°C and the resulting supernatant was heat-treated in a hot water bath at 90 °C 00:15:00 .
- 4 Insoluble material was separated by centrifugation at 13000 x g, 4°C, 00:10:00 , and the supernatant, containing the enzyme, was analyzed on a 12% SDS–polyacrylamide.
- 5 The purified elution fractions were dialysed Overnight 50 millimolar (mM) 7 4 °C .