

# Single-step purification by heat shock

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**1** Works for me [dx.doi.org/10.17504/protocols.io.bgbsjsne](https://doi.org/10.17504/protocols.io.bgbsjsne)

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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 NaH<sub>2</sub>PO<sub>4</sub>, 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.

## EXTERNAL LINK

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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 Milimolar (mM) NaH<sub>2</sub>PO<sub>4</sub>** ,  **300 Milimolar (mM) NaCl** ,  **10 Milimolar (mM) Imidazole** and  **0.05 % volume Tween 20** at  **pH7** )
- 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 Milimolar (mM)**  **pH7**  **4 °C** .