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# Protein purification

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[1]iGEM Gifu

protocol .

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The extraction method is based on the paper by Janice S. Chen et al. The cultured E. coli is collected by centrifugation and then disrupted by sonication. The extracted solution is purified by affinity chromatography using a Ni-NTA column with an x10 His tag attached to Cas12a. Clontech's His60 Ni Gravity Column Purification Kit is used for purification, and the experimental method is based on the kit protocol.

Yuichiroh Ikagawa 2021. Protein purification. **protocols.io** https://protocols.io/view/protein-purification-bzcsp2we

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**Reagents**

PBS

Lysis buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% (v/v) glycerol, 0.25 mg/ml lysozyme)

His60 Ni Gravity Column Purification Kit (Clontech Laboratories, Inc.)

- Equilibrium buffer (50 mM sodium phosphate, 300 mM sodium chloride, 20 mM imidazole; pH 7.4)
- Wash buffer (Elution buffer: 710 µl, Equilibration Buffer: 9.29 ml)
- Elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole; pH 7.4 )

**Equipment**

Sonicator

His60 Ni Gravity Column (Clontech Laboratories, Inc.)

extraction

1 Transfer 50 ml of culture medium to a 50 ml tube.

2 Collect the pellet by centrifugation at 5,000 g for 5 minutes 🌡 **4 °C**

3 Resuspend the pellet with 2 ml PBS.

4 Dispense the culture medium into 1.5 ml tubes.

5 Centrifuge at 15,000 g for 30 minutes.

6 Resuspend 500 µl Lysis buffer.

7 Disrupt the cell by sonication (x10 sonication: 10 seconds/incubation: 10 seconds) on ice.

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8 Fully suspend the matrix before opening the column. 🌡 **4 °C**

9 Carefully remove the bottom stopper.

10 Add 1ml of Equilibration buffer to the His60 Ni gravity column 10 times. 🌡 **4 °C**

11 Attach the bottom stopper.

12 Add cell extraction sample to the column. **4 °C**

13 Carefully connect the top stopper to the top of the column.

14 Slowly Inverting the column for 1 hour. **4 °C**

15 Install the column in a vertical position and let the resin settle at the bottom of the column. **4 °C**

16 Carefully remove the top stopper.

17 Remove the bottom stopper and collect 1 ml fraction each into 1.5 ml tubes. **4 °C**

18 Add 1 ml Equilibration buffer to the column 10 times. **4 °C**

19 Add 1 ml Wash Buffer to the column 10 times. **4 °C**

20 Add Elution Buffer to the column and collect 1 ml fractions. **4 °C**

21 Measure the absorbance 280 nm of the fractions by nanodrop.

22 Added 1 ml Equilibration Buffer to the column 20 times. **4 °C**

23 Wash the column with 1 ml of water 5 times **4 °C**

24 Attach the bottom stopper.

25 Add 20% ethanol to the column for storage. **4 °C**

26 Attach top stopper and storage. **4 °C**