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Protocol for batch/gravity-flow purification of Histidine-tagged recombinant proteins under native conditions using Ni Sepharose 6 Fast Flow and Empty Disposable PD-10 Columns

Recommended buffers*

Buffer A (Binding/wash buffer): 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.3

Buffer B (Elution buffer): 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.3

* The buffers above are recommended for binding, wash, and elution of Histidine-tagged recombinant proteins using batch/gravity-flow procedures. However, the imidazole concentration may need to be optimized for each individual Histidine-tagged protein to ensure the best balance of purity (low binding of unwanted proteins), and yield (binding of all of the Histidine-tagged protein). For most Histidine-tagged proteins, an imidazole concentration of 20–40 mM in the binding and wash buffers is a good starting point. Note that Ni Sepharose™ 6 Fast Flow often requires a slightly higher concentration of imidazole in the binding buffer than other IMAC (Immobilized Metal Ion Adsorption Chromatography) media on the market.

Sample preparation

1. Harvest host cells with expressed Histidine-tagged protein according to manufacturers' recommendations.
2. Lyse host cells by, for example, sonication in 5 ml of Buffer A (Binding/wash buffer, see Recommended buffers)/g cell paste.
3. Clarify lysate by centrifugation at 10 000 × g for 10 min.
4. Filter lysate through a 0.45-µm filter.

Preparation of Ni Sepharose 6 Fast Flow

1. Gently shake the bottle in which the Ni Sepharose 6 Fast Flow is supplied until the medium is homogeneous.
2. Remove 2 ml slurry from the bottle and transfer to a centrifuge tube.
3. Sediment the Ni Sepharose 6 Fast Flow by centrifugation at 500 × g for 5 min.
4. Discard supernatant and replace with 5 ml of distilled water.
5. Gently shake the Ni Sepharose 6 Fast Flow for 3 min and resediment by centrifugation at 500 × g for 5 min.
6. Repeat steps 4 and 5 using Buffer A instead of distilled water.
7. Transfer the Ni Sepharose 6 Fast Flow to a measuring cylinder.
8. Add an appropriate volume of Buffer A to make a 50% slurry.

Sample binding

1. Add 4 ml of sample to 1 ml of the 50% slurry. Binding capacity of Ni Sepharose 6 Fast Flow is protein dependent and the average is 40 mg/ml. This means that 1 ml of the 50% slurry can bind about 20 mg of Histidine-tagged protein.
2. Incubate sample and the Ni Sepharose 6 Fast Flow slurry at room temperature on a shaker at low speed for 1 h.

Preparation of the Empty Disposable PD-10 Columns

1. Wash the filter with 20% ethanol.
2. Rinse the filter with distilled water.
3. Insert the filter into the Empty Disposable PD-10 Column.

Buffer wash and elution

1. Load sample/Ni Sepharose 6 Fast Flow mix onto the PD-10 columns and collect flowthrough.
2. Wash with 2 × 1 ml of Buffer A and collect both fractions.
3. Elute with 4 × 0.5 ml of Buffer B (Elution buffer, see Buffers required) and collect the eluted fractions in four separate tubes.
4. Measure absorbance at 280 nm using a spectrophotometer and confirm purity of pooled fractions by SDS-PAGE. Use Buffer B as the blank.

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