Protocol-at-a-Glance

TALON® Single Step Column (5 ml) Protocol-at-a-Glance

(PT3755-2)

The **TALON Single Step Columns** can be used with either gravity flow or centrifuge purification protocols. For purifications of less than 10 samples, we recommend using the gravity flow procedure. For high throughput purification of more than 10 samples, the centrifuge procedure should be faster.

1. Buffer Preparation and Additional Materials Required

- Equilibration/Wash Buffer (50 mM Sodium Phosphate, 300 mM NaCl, pH 7.0)
- Wash-2 Buffer (50 mM Sodium Phosphate, 300 mM NaCl, 7.5 mM Imidazole, pH 7.0)*
- Elution Buffer (50 mM Sodium Phosphate, 300 mM NaCl, 150 mM Imidazole, pH 7.0)

* **Note:** The Wash-2 Buffer can be made by mixing 1 part Elution Buffer with 19 parts Equilibration/Wash Buffer.

- 15 ml Falcon Tubes, receiving tubes for fraction storage.
- Bio-Rad Protein Assay (Bio Rad Cat. No. 500-0001)

2. Sample preparation and lysis

a. TALON Single Step Columns can be used for purification of any His-tagged protein from an *E. coli* culture. For example, if screening transformants for expression levels, pick a single colony from the plate and inoculate 4.7 ml of medium. Incubate the culture at 37°C until the OD_{600} reaches ~0.6–0.8 AU (entering log phase). Then induce protein expression with the recommended concentration of inducer agent (depending on your expression strain and the expression plasmid being used). Continue to grow the culture with rigorous shaking at 37°C for another 2–4 hours. Alternatively, follow your standard induction or expression protocol. [Optional: Remove 200 µl of the expression culture for SDS-PAGE analysis.]

b. Place the bottom closure firmly on a TALON Single Step Column, remove the top cap, add 4.5 ml of culture and then replace the top cap. Mix the suspension either on a carousel shaker for 20–30 min at room temperature or by inverting the tube every 2 min for a total of 30 min.

c. Remove the top cap and the bottom closure and place the Talon Single Step Column into a Receiving Tube. Proceed with either the gravity flow or the centrifuge procedure.

3. Gravity Flow Procedure

a. Let the extract drain by gravity flow. Remove the column from the receiving tube and replace the bottom closure. [Optional: Remove 200 µl of non-adsorbed material from the Receiving Tube for SDS-PAGE and Protein Assay analysis (Step 4).]

b. Add 4.5 ml of Equilibration/Wash Buffer, replace the top cap and resuspend the resin by inverting the column. Remove the bottom closure and put the column into a Receiving Tube. Allow the wash to drain by gravity flow. [Optional: Remove 200 µl of Wash-1 from the Receiving Tube for SDS-PAGE and Protein Assay analysis.]

c. [Optional] For improved purity of target protein, repeat Step b twice.

d. Replace the bottom closure, then add 4.5 ml of Wash-2 Buffer. Replace the top cap and resuspend the resin by inverting the column. Remove the bottom closure and put column into a Receiving Tube. Let the buffer drain by gravity flow. [Optional: Remove 200 µl of Wash-2 from the Receiving Tube for SDS-PAGE and Protein Assay analysis.]

e. [Optional] For improved purity of the target protein, repeat the wash in Step d twice.

f. Add 1 ml of Elution Buffer and resuspend the resin by inverting the columns for 2 min.

g. Remove the bottom closure and put the column into a Receiving Tube. Allow the elution fraction to drain by gravity flow. Proceed with Step 5. Protein Analysis.

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4. Centrifuge Procedure

a. Centrifuge at 700 x g for 2 min. Take the column from the Receiving Tube and replace the bottom closure. [Optional: Remove 200 µl of non-adsorbed material collected in the Receiving Tube for SDS-PAGE and Protein Assay analysis.]

b. Add 4.5 ml of Equilibration/Wash Buffer, replace the top cap and resuspend the resin by inverting the column. Remove the bottom closure and put the column into a Receiving Tube. Centrifuge at 700 x g for 2 min. Remove the column from the Receiving Tube and replace the bottom closure. [Optional: Remove 200 µl of Wash-1 for SDS-PAGE and Protein Assay analysis.]

c. [Optional] For improved purity of target protein, repeat Step b twice.

d. Add 4.5 ml of Wash-2 Buffer, replace the top cap and resuspend the resin by inverting the column. Remove the bottom closure and put the TALON Single Step Column into a Receiving Tube. Centrifuge at 700 x g for 2 min. Remove the column from the Receiving Tube and replace the bottom closure. [Optional: Remove 200 µl of Wash-2 from the Receiving Tube for SDS-PAGE and Protein Assay analysis.]

e. [Optional] For improved purity of the target protein, repeat the wash in Step d twice.

f. Add 1 ml of Elution Buffer and resuspend the resin by inverting the column for 2 min.

g. Remove the bottom closure and put the TALON Single Step Column into a Receiving Tube. Centrifuge the column in the tube at 700 x g for 2 min. Proceed with Step 5. Protein Analysis.

5. Protein Analysis

Determine amount of protein in a 1:10 dilution of the non-adsorbed fractions and the amount of protein in the (undiluted) elution fraction by performing a Bio-Rad Protein Assay. Analyze the samples by SDS-PAGE to determine the purity of the target protein (Elution fraction).

This document has been reviewed and approved by the Clontech Quality Assurance Department.