Protocol-at-a-Glance

# TALON® Single Step Column (20 ml) Protocol-at-a-Glance

**(PT3788-2)**

TALON Single Step Columns can be used for purification of any polyhistidine-tagged protein from an *E. coli* culture—with either a gravity flow protocol (faster for <10 samples) or centrifuge purification protocol (faster for ≥10 samples).

## 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.

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


## 2. Sample Preparation and Lysis

a. Express your protein in 25 ml of bacterial culture.

b. Seat end cap firmly on the TALON Single Step Column, remove top cap, add 20 ml culture and then replace 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 end cap and return the column to the receiving tube. Proceed with either the gravity flow (Step 3) or the centrifuge (Step 4) procedure.


## 3. Gravity Flow Procedure

a. Let the extract drain by gravity flow. Remove the column from the receiving tube and replace end cap.

b. Add 20 ml Equilibration/Wash Buffer, place column in a fresh receiving tube, replace top cap, and invert to resuspend resin. Remove end cap and return column to the receiving tube. Allow wash to drain by gravity flow. **[Optional] For improved purity, repeat this step twice.**

c. Replace the end cap, put the column into a fresh receiving tube, then add 20 ml Wash-2 Buffer. Replace top cap and invert to resuspend resin. Remove end cap and return the column to the Receiving Tube. Let the buffer drain by gravity flow. **[Optional] For improved purity, repeat this step twice.**

d Replace the end cap, add 2.0 ml Elution Buffer, place the 20-ml column into a fresh receiving tube, and invert for 2 min to resuspend resin. For an additional 10–15% of purified protein, repeat elution with an additional 2.0 ml Elution Buffer.

e. Remove the end cap and return column to the receiving tube. Allow the elution fraction to drain by gravity flow to obtain the final sample.


## 4. Centrifuge Procedure

a. Centrifuge the column (2 min at 700 x g), then remove it from the receiving tube and replace end cap.

b. Place column in a fresh receiving tube, then add 20 ml of Equilibration/Wash Buffer, replace top cap, and invert to resuspend resin. Remove end cap, return column to receiving tube, and centrifuge (2 min at 700 x g). **[Optional] For improved purity, repeat this step twice**.

c. Remove the column from the receiving tube and replace the end cap. Place the column in a fresh receiving tube, add 20 ml Wash-2 Buffer, replace top cap and invert to resuspend resin. Remove end cap. return the Single Step Column to the receiving tube, and centrifuge (2 min at 700 x g). **[Optional] For improved purity, repeat this step twice**.

d. Remove column from the receiving tube and replace the end cap. Add 2 ml Elution Buffer, place column in a fresh receiving tube, close top cap, and invert for 2 min to resuspend resin. For an additional 10-15% of purified protein, repeat elution with an additional 2.0 ml Elution Buffer.

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Clontech

**United States/Canada**
800.662.2566
**Asia Pacific**
+1.650.919.7300
**Europe**
+33.(0)1.3904.6880
**Japan**
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

e. Remove end cap, return column to the receiving tube, and centrifuge (2 min at 700 x g) to obtain the final sample.

**5. Protein Analysis**

Determine the 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).