

TALON® Metal Affinity Resins

Protocol-at-a-Glance

(PT1320-2)

Please read the *User Manual* before using this *Protocol-at-a-Glance*. This summary for purifying polyhistidine-tagged proteins with TALON®, TALON® Superflow, TALON® CellThru, and TALONspin™ Columns is provided for your convenience and is not intended for first-time users. For medium-pressure column purification using TALON Superflow, please see Section VII.E of the *User Manual*.

Method	Spin column (Section VII.F)	Batch (Section VII.D)	Batch/gravity-flow (Section VII.C)
Binding conditions			
Resin bed-volume ^a	0.5 ml	1.0 ml	1.0 ml
polyhistidine-protein binding capacity	2–3 mg	~5 mg	~5 mg
Sample volume	1 ml (each loading)	~13 ml	~13 ml
Binding time	5 min	20 min	20 min
Washing conditions^{b, c}			
pH gradient	2 x 1 ml (pH 7)	2 x 10 ml (pH 7)	2 x 10 ml (pH 7)
	1 x 1 ml (pH 7) (optional)	1 x 10 ml (pH 7)	2 x 3 ml (pH 7)
Imidazole	2 x 1 ml (pH 7) + 5–10 mM imid.	2 x 5 ml (pH 7) + 5–10 mM imid.	2 x 3 ml (pH 7) + 5–10 mM imid (in column)
Washing time	5 min/wash	10 min/wash	10 min/wash
Elution conditions			
pH gradient	2 x 0.5 ml (pH 5) (1-ml fractions)	4 x 1 ml pH (5) (0.5-ml fractions)	4 x 1 ml (pH 5)
Imidazole	2 x 0.5 ml (pH 7) + 100 mM imid. (1-ml fractions)	4 x 1 ml (pH 7) + 100 mM imid. (0.5-ml fractions)	4 x 1 ml (pH 7) + 100 mM imid.
Elution time	5 min/elution	10 min/elution	10 min/elution

^a Volume of Talon Resin that was prepared as described in the *User Manual*, Section VII.C.1–7.

^b "2 x 1 ml (pH 7.0)" means wash two times with 1 ml of buffer at pH 7.0 each time.

^c We recommend performing your first purification at pH 7; however, if your target protein is more stable at pH 8, then use the Extraction Buffer (pH 8) in place of the Extraction/Wash Buffer (pH 7) during all extraction, wash, and elution steps.

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