

HisTALON™ Gravity Column Purification Kit Protocol-at-a-Glance

(PT4435-2)

Please read the *User Manual* before using this *Protocol-at-a-Glance*. This summary for purifying his-tagged proteins with HisTALON Gravity Columns is provided for your convenience and is not intended for first-time users.

Binding Conditions

Resin bed-volume	1.0 ml
Polyhistidine-protein binding capacity	up to 20 mg/ml; depending on individual protein

Method	Gravity-Flow
Column Equilibration	
Equilibration buffer	5-10 ml
Elapsed time	10 min
Load Column	
Load clarified sample onto column	Collect 1 ml fractions
Elapsed time	5-10 min; depending on sample volume
Column Washes	
Equilibration buffer	8 ml
Wash buffer, 10 mM imidazole (optional)	7 ml (1 part Elution buffer, 14 parts Equilibration buffer)
Elapsed time	15 min
Elution	
Elution buffer, 150 mM imidazole	5-8 ml (Monitor by absorbance at 280 nm or Bradford Assay)
Elapsed time	10 min



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

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