



# Total solutions for preparation of histidine-tagged proteins

Selection Guide



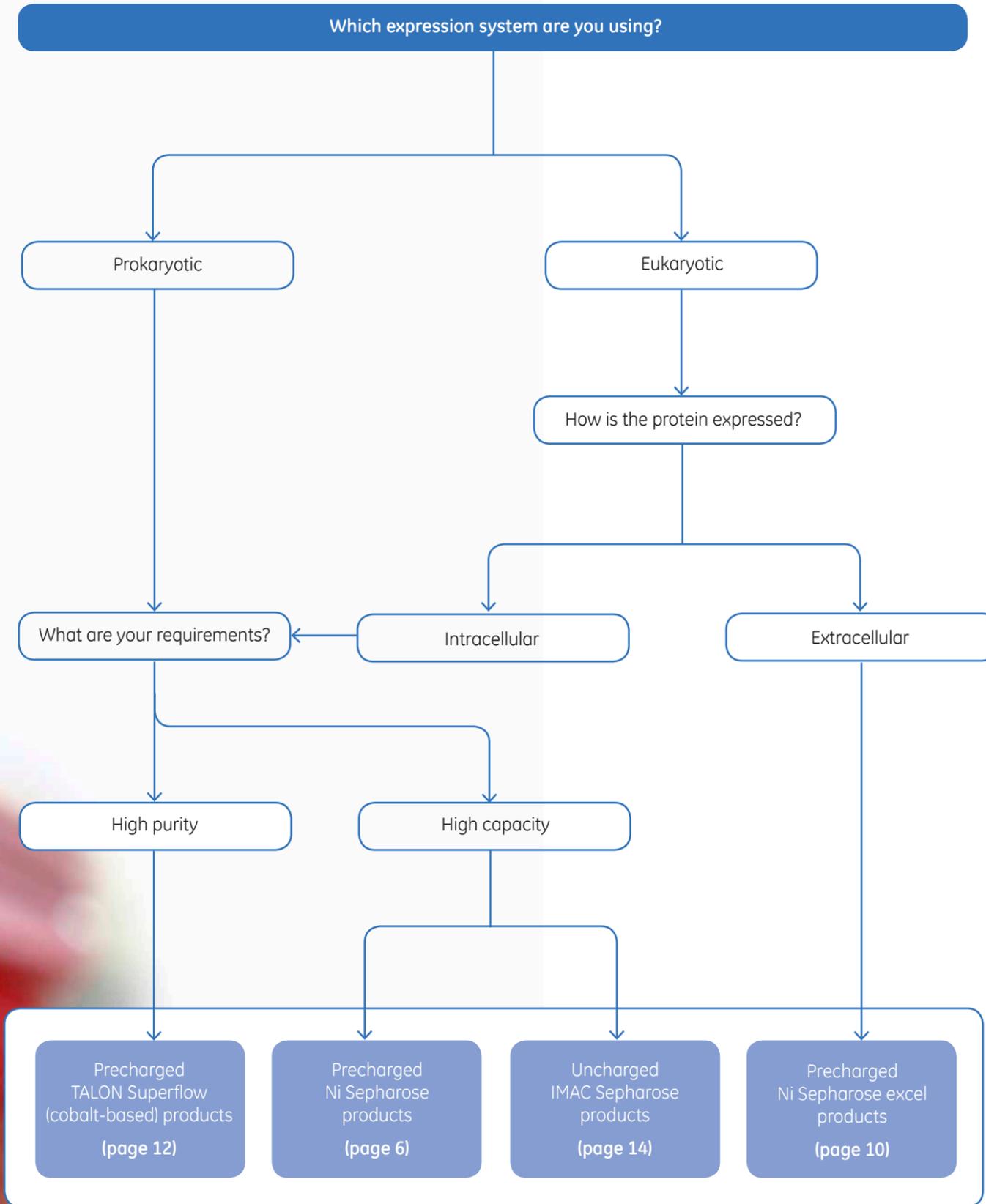
GE Healthcare Life Sciences offers a wide range of products for convenient and flexible purification of histidine-tagged proteins produced in prokaryotic and eukaryotic expression systems. Five categories of chromatography media (resins) are available—Ni Sepharose™ High Performance (HP), Ni Sepharose 6 Fast Flow (FF), His Mag Sepharose Ni, which are precharged with nickel ions, TALON® Superflow™ (precharged with cobalt ions), Ni Sepharose excel, His Mag Sepharose excel (precharged with nickel ions very strongly bound to the chelating ligand), and uncharged IMAC Sepharose HP and IMAC Sepharose 6 FF. These media are available in a range of prepacked formats for convenience.

GE Healthcare's products for histidine-tagged protein purification offer the following benefits:

- Flexible choice of uncharged or nickel/cobalt precharged media
- High protein binding capacity with low leakage of nickel or cobalt ions
- Range of products covers purification of tagged proteins from prokaryotic and eukaryotic expression systems
- Different formats allow screening, capture purification, and scale-up

Use the workflow on page 5 to locate the right product for your needs on the pages of this guide.

# Select your product category



# Precharged Ni Sepharose products



Ni Sepharose High Performance (HP), Fast Flow (FF), and His Mag Sepharose Ni media are precharged with Ni<sup>2+</sup> ions for convenience. These media provide high binding capacity combined with lower leakage of Ni<sup>2+</sup> ions compared to products from other suppliers\*, which ensures reliable capture of histidine-tagged proteins with high repeatability between runs.

**Ni Sepharose HP** has a high protein binding capacity and the small bead size ensures high-performance purification and minimal sample dilution. Ni Sepharose HP is available in lab packs and prepacked HisTrap™ HP columns, as well as His SpinTrap™ columns for small-scale purification (minipreps) by centrifugation and His MultiTrap™ HP 96-well plates for high-throughput screening and parallel purification.

**Ni Sepharose 6 FF** has high protein binding capacity, which converts to lower costs per prep as a result of the lower medium requirement. The flow properties of the highly cross-linked agarose matrix make it an excellent choice for purification scale-up.

Ni Sepharose 6 FF is available in bulk packs for batch purification and scale-up. The medium is also available in prepacked His MultiTrap FF 96-well filter plates for screening purposes and His GraviTrap™ columns for convenient and reproducible gravity-flow preparations. To enable reliable scale-up, Ni Sepharose 6 FF is available in HisTrap FF columns that can be connected in series, as well as larger HisPrep™ FF 16/10 columns when greater capacity is needed. Purification can be performed directly from unclarified lysates using HisTrap FF crude, His GraviTrap, His SpinTrap, and His MultiTrap columns and 96-well plates. This allows fast purifications and reduces sample preparation time, which minimizes degradation of sensitive target proteins by proteases present in the cell lysate. For process development, prepacked HiScreen™ Ni FF columns with 10 ml bed heights are used. These columns can be connected in series for increased capacity.

**His Mag Sepharose Ni** consists of Sepharose based magnetic beads designed for small-scale purification and expression screening of histidine-tagged proteins. These visible and dense beads allow convenient capture and simplified handling of histidine-tagged proteins using a magnetic rack.

**Ni Sepharose excel** and **His Mag Sepharose excel** are IMAC media designed for screening and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. Find out more about Ni Sepharose excel and His Mag Sepharose excel on page 10.

\* See Data files 18-1174-40, 11-0008-86, 28-4041-05, 28-9797-23, and 28-4041-06 for details.

## Technical overview

Product	Product code no.	Data file code no.	Pack size	Approx. protein binding capacity*	High-throughput screening	Minipreps	Batch/Gravity flow	Syringe compatible	Scale-up	AKTA <sup>†</sup> system compatibility	Process development	Magnetic separation	
Ni Sepharose High Performance	17-5268-01 17-5268-02	18-1174-40	25 ml 100 ml	40 mg/ml	•							•	
His MultiTrap HP	28-4009-89	11-0036-63	4 × 96-well filter plates	1000 µg/well	•	•							
His SpinTrap	28-4013-53	28-4046-59	50 × 100 µl	750 µg/column	•	•							
His SpinTrap Kit <sup>‡</sup>	28-9321-71	28-4046-59	50 × 100 µl	750 µg/column	•	•							
HisTrap HP	17-5247-01 17-5247-05	18-1174-40	5 × 1 ml 100 × 1 ml <sup>§</sup>	40 mg/column								•	
	17-5248-01 17-5248-02 17-5248-05		1 × 5 ml 5 × 5 ml 100 × 5 ml <sup>§</sup>	200 mg/column								•	
												•	
Ni Sepharose 6 Fast Flow	17-5318-06 17-5318-01 17-5318-02 17-5318-03	11-0008-86	5 ml 25 ml 100 ml 500 ml	40 mg/ml	•	•	•	•	•	•			
						•	•	•	•	•			
						•	•	•	•	•	•		
						•	•	•	•	•	•		
His Mag Sepharose Ni	28-9673-88 28-9673-90 28-9799-17		2 × 1 ml 5 × 1 ml 10 × 1 ml	50 mg/ml	•							•	
					•							•	
					•							•	
His MultiTrap FF	28-4009-90	11-0036-63	4 × 96-well filter plates	800 µg/well	•	•							
His GraviTrap	11-0033-99	11-0036-90	10 × 1 ml	40 mg/column			•						
His GraviTrap Kit <sup>‡</sup>	28-4013-51	11-0036-90	20 × 1 ml	40 mg/column			•						
HisTrap FF	17-5319-01 17-5319-02	11-0008-86	5 × 1 ml 100 × 1 ml <sup>§</sup>	40 mg/column				•		•			
	17-5255-01 17-5255-02		5 × 5 ml 100 × 5 ml <sup>§</sup>	200 mg/column					•		•		
HisTrap FF crude	11-0004-58 11-0004-59	11-0012-37	5 × 1 ml 100 × 1 ml <sup>§</sup>	40 mg/column				•		•			
	17-5286-01 17-5286-02		5 × 5 ml 100 × 5 ml <sup>§</sup>	200 mg/column					•		•		
HisTrap FF crude Kit <sup>‡</sup>	28-4014-77	11-0012-37	3 × 1 ml	40 mg/column				•					
HiScreen Ni FF	28-9782-44	28-9305-81	1 × 4.7 ml	40 mg/column						•	•	•	
HisPrep FF 16/10	28-9365-51	11-0008-86	1 × 20 ml	800 mg/column					•	•			
<b>Buffer kit</b>													
His Buffer Kit	11-0034-00	11-0036-90				•	•	•					

\* Protein-dependent

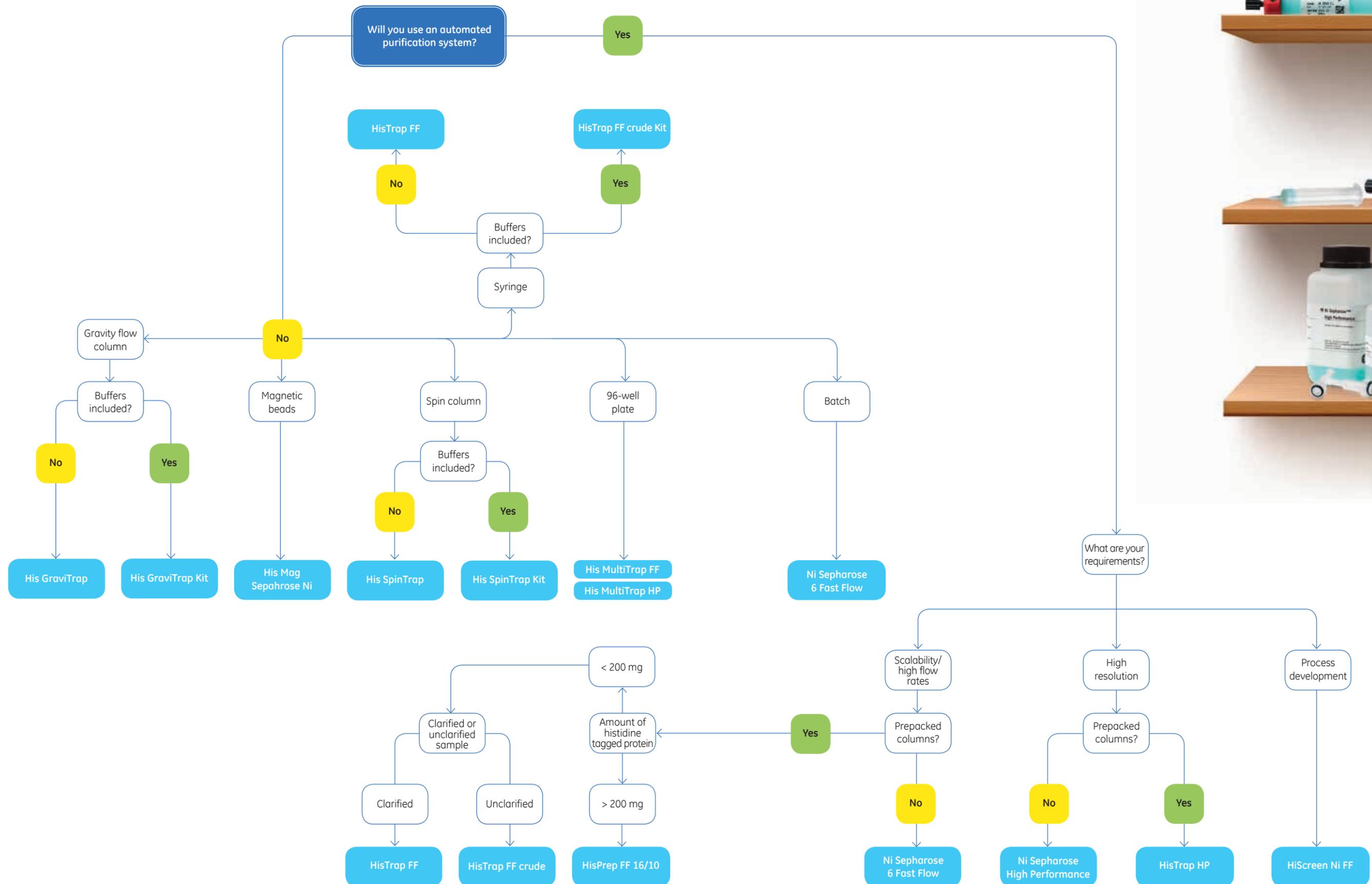
<sup>†</sup> Includes buffer stock solutions for approximately 10 preps using a syringe

<sup>‡</sup> Includes buffer stock solutions for approximately 20 preps

<sup>§</sup> Includes buffer stock solutions for approximately 50 preps

<sup>¶</sup> Available by specific customer order

# Selecting Ni Sepharose products for manual/automated purification



# Ni Sepharose excel products

Purification from eukaryotic cell culture supernatants



Traditionally, IMAC purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants often leads to stripping of the immobilized metal ions during sample application. This results in low or no binding of the target protein. Purification is further complicated by the fact that the target protein concentration is low, which results in large sample volumes. To overcome these problems, extensive sample pretreatment is required, such as buffer exchange by dialysis in combination with concentration procedures. Such pretreatment is often time-consuming and potentially harmful to sensitive proteins.

**Ni Sepharose excel** is a novel IMAC medium precharged with nickel ions that are bound very strongly. Ni Sepharose excel is designed primarily for capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants, such as from insect cells or CHO cells. The medium is also suitable for purification of histidine-tagged proteins originating from other samples causing nickel stripping. Ni Sepharose excel is available in lab packs for scale-up and packing in chromatography columns such as Tricorn™, XK, or HiScale™ columns as well as in prepacked HisTrap excel columns, which allow purification of unclarified lysates when used with chromatography systems.

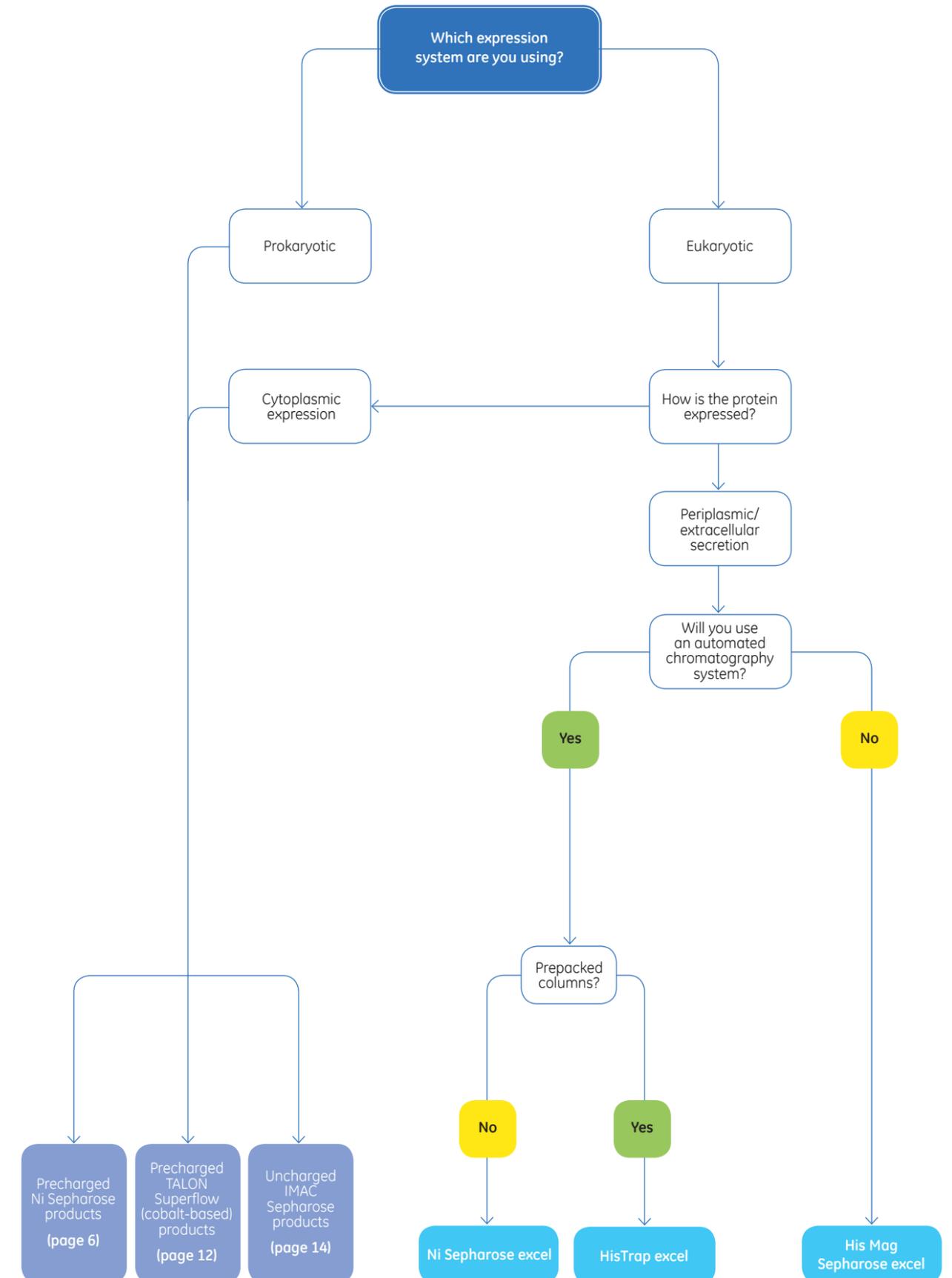
**His Mag Sepharose excel** are Sepharose based magnetic beads designed for small-scale purification and expression screening of histidine-tagged proteins. These visible and dense beads allow convenient capture and handling of histidine-tagged proteins using a magnetic rack.

## Technical overview

Product	Product code no.	Data file code no.	Pack size	Approx. protein binding capacity*	High-throughput screening	Minipreps	Batch/Gravity flow	Syringe compatible	Scale-up	AKTA system compatibility
Ni Sepharose excel	17-3712-01	29-0168-49	25 ml	10 mg/ml	•	•	•	•	•	•
	17-3712-02		100 ml		•	•	•	•	•	
	17-3712-03		500 ml		•	•	•	•	•	
HisTrap excel	17-3712-05	29-0168-49	5 × 1 ml	10 mg/column 50 mg/column			•			•
	17-3712-06		5 × 5 ml							
His Mag Sepharose excel	17-3712-20	29-0168-49	2 × 1 ml	10 mg/ml	•	•				
	17-3712-21		5 × 1 ml		•	•				
	17-3712-22		10 × 1 ml		•	•				

\* Protein-dependent

## Selecting Ni Sepharose excel products



# Precharged TALON Superflow (cobalt-based) products



**TALON Superflow** is a cobalt-based IMAC medium offering an alternative selectivity compared to nickel-charged media. It is the first-choice precharged medium when target protein purity is more important than target protein yield. TALON Superflow binds polyhistidine-tagged proteins with enhanced selectivity and exhibits a reduced affinity for host proteins giving lower background. The medium is suitable for IMAC purification of histidine-tagged proteins when Ni<sup>2+</sup> is not the optimal choice of metal ion. It is appropriate for purifying proteins under native or denaturing conditions and allows use of imidazole concentrations in sample, wash and elution buffers that are typically lower than those used with nickel-based media.

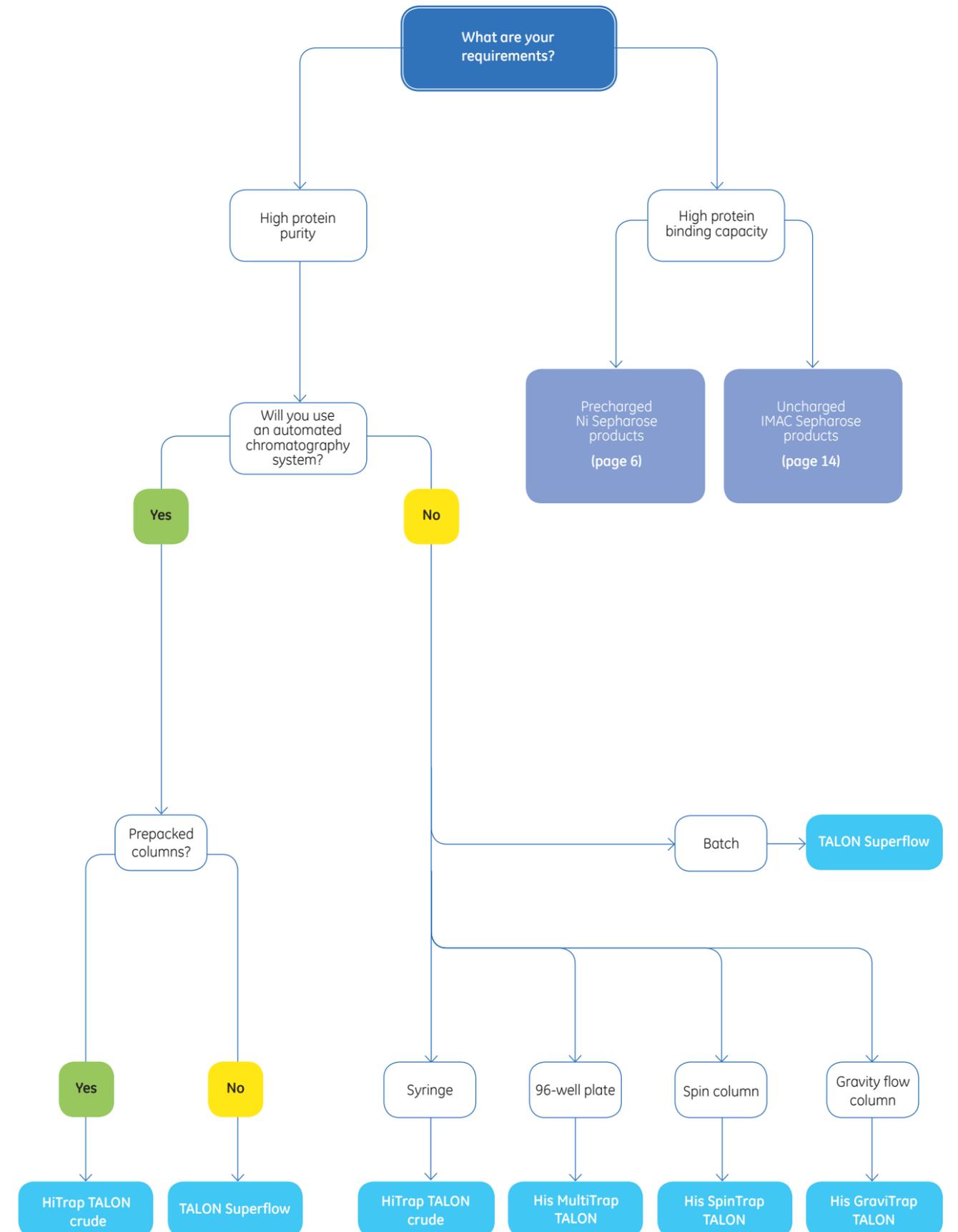
TALON Superflow is available in lab packs for batch purification and scale-up and in versatile prepacked formats including His MultiTrap TALON 96-well filter plates for screening/parallel purification, His SpinTrap TALON for minipreps, and His GraviTrap TALON columns for reproducible gravity-flow preparations. HisTrap TALON crude columns enable direct loading of unclarified cell lysates for fast and convenient purification of histidine-tagged proteins.

## Technical overview

Product	Product code no.	Data file code no.	Pack size	Approx. protein binding capacity*	High-throughput screening	Minipreps	Batch/Gravity flow	Syringe compatible	Scale-up	ÅKTA system compatibility
TALON Superflow	28-9574-99 28-9575-02	28-9664-10	10 ml 50 ml	20 mg/ml 20 mg/ml	•	•	•	•		
His MultiTrap TALON	29-0005-96	28-9664-10	4 × 96-well plates	up to 1 mg/well	•	•				
His SpinTrap TALON	29-0005-93	28-9664-10	50 × 100 µl	up to 1 mg/column	•	•				
His GraviTrap TALON	29-0005-94	28-9664-10	10 × 1 ml	up to 15 mg/column			•			
HiTrap™ TALON crude	28-9537-66 28-9538-05 28-9537-67 28-9538-09	28-9664-10	5 × 1 ml 100 × 1 ml† 5 × 5 ml 100 × 5 ml†	20 mg/column 20 mg/column 100 mg/column 100 mg/column				•	•	•

\* Protein-dependent  
† Available by specific customer order

## Selecting TALON Superflow products for manual/automated purification



# Uncharged IMAC Sepharose products



Immobilized metal ion affinity chromatography (IMAC) exploits a molecule's affinity for chelated metal ions. The amino acid histidine present in many proteins forms complexes with transition metal ions such as  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Fe}^{3+}$ . Media with a suitable immobilized metal ion will therefore selectively retain proteins with exposed histidine residues. Exposed cysteine and tryptophan residues may also be involved in the binding to an immobilized metal ion but their contribution to the binding is much lower than the contribution from exposed histidine residues.

**IMAC Sepharose HP** medium is supplied free of metal ions. The medium can be charged with a metal ion of choice, which allows screening to select the most suitable metal ion and purification conditions for specific histidine-tagged and untagged target proteins. IMAC Sepharose HP ensures low sample dilution and excellent separation. The small bead size of the Sepharose HP matrix allows high-performance purifications. The medium is available in lab packs and in prepacked HiTrap IMAC HP columns.

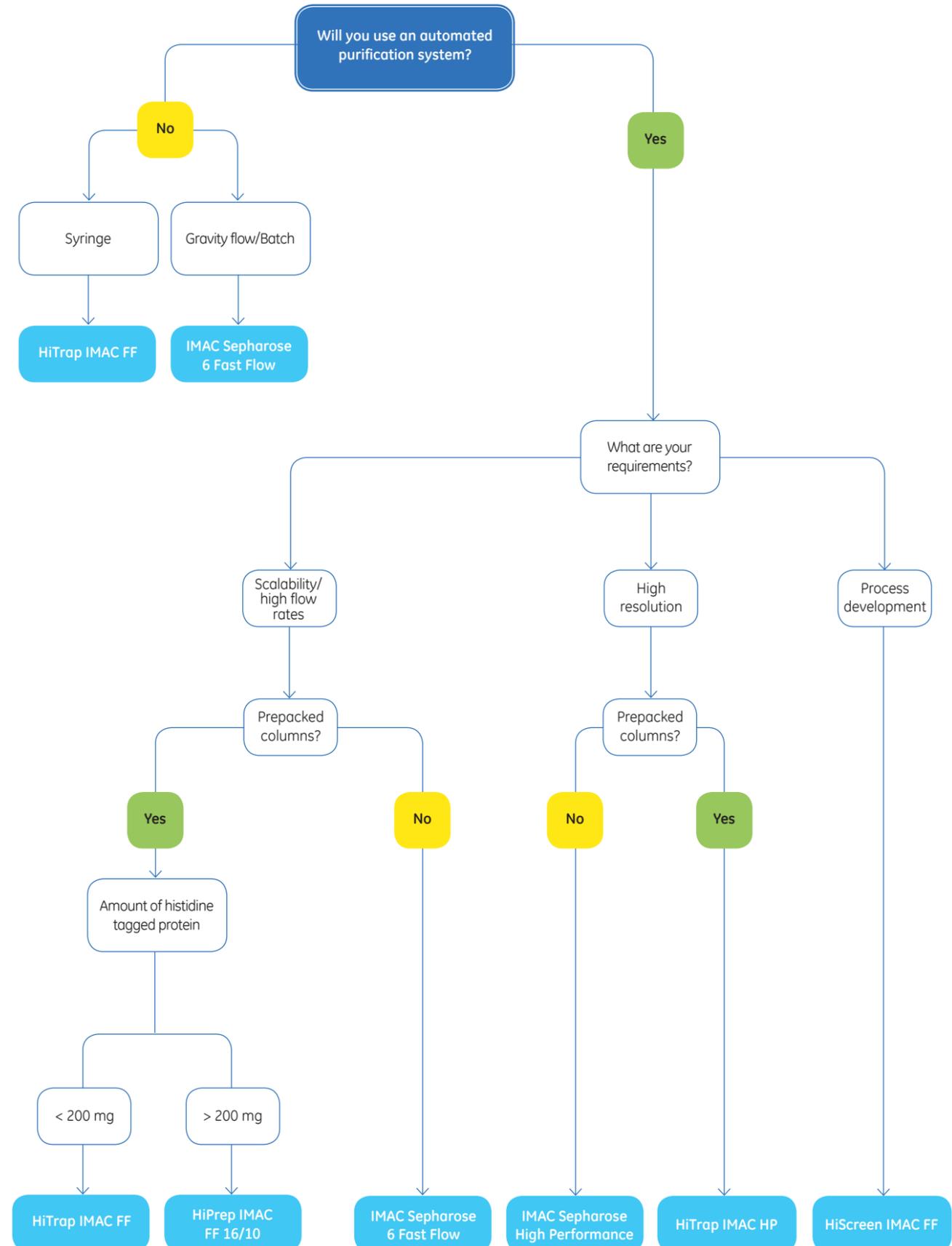
**IMAC Sepharose 6 FF** is an uncharged medium for screening for optimal purification conditions and scaling up purification of histidine-tagged and untagged proteins. The medium is supplied ready for charging with metal ions. The excellent flow properties of IMAC Sepharose 6 FF allow easy scale-up and the medium is also available in convenient, prepacked HiTrap IMAC FF columns. Further scale-up can be achieved with HiPrep™ IMAC FF 16/10, 20 ml columns. For process development, prepacked HiScreen IMAC FF columns with 10 ml bed heights are used. These columns can be connected in series for increased capacity.

## Technical overview

Product	Product code no.	Data file code no.	Pack size	Approx. protein binding capacity*	High-throughput screening	Minipreps	Batch/Gravity flow	Syringe compatible	Scale-up	AKTA system compatibility	Process development
<b>Uncharged IMAC Sepharose</b>											
IMAC Sepharose High Performance	17-0920-06 17-0920-07	28-4041-05	25 ml 100 ml	40 mg/ml ( $\text{Ni}^{2+}$ ) <sup>*</sup>	•					•	•
HiTrap IMAC HP	17-0920-03 17-0920-05	28-4041-05	5 × 1 ml 5 × 5 ml	40 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup> 200 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup>						•	•
IMAC Sepharose 6 Fast Flow	17-0921-07 17-0921-08	28-4041-06	25 ml 100 ml	40 mg/ml ( $\text{Ni}^{2+}$ ) <sup>*</sup> 25 mg/ml ( $\text{Cu}^{2+}$ ) <sup>*</sup> 15 mg/ml ( $\text{Zn}^{2+}$ ) <sup>*</sup>	•	•	•			•	•
HiTrap IMAC FF	17-0921-02 17-0921-04	28-4041-06	5 × 1 ml 5 × 5 ml	40 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup> 200 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup> 125 mg/column ( $\text{Cu}^{2+}$ ) <sup>*</sup> 75 mg/column ( $\text{Zn}^{2+}$ ) <sup>*</sup>				•	•	•	
HiPrep IMAC FF 16/10	28-9365-52	28-4041-06	1 × 20 ml	800 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup> 500 mg/column ( $\text{Cu}^{2+}$ ) <sup>*</sup> 300 mg/column ( $\text{Zn}^{2+}$ ) <sup>*</sup>						•	•
HiScreen IMAC FF	28-9505-17	28-9305-81	1 × 4.7 ml	40 mg/column ( $\text{Ni}^{2+}$ ) <sup>*</sup> 15 mg/column ( $\text{Zn}^{2+}$ ) <sup>**</sup> 25 mg/column ( $\text{Cu}^{2+}$ ) <sup>**</sup>						•	•
<b>Buffer kit</b>											
His Buffer Kit	11-0034-00	11-0036-90				•	•	•			

\* ( $\text{Histidine}$ )<sub>n</sub>-tagged proteins, protein-dependent  
 \*\* Untagged protein, protein-dependent

## Selecting IMAC Sepharose products for manual/automated purification



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**IMAC Sepharose products and Ni Sepharose products**  
Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent numbers 5,284,933 and 5,310,663, and equivalent patents and patent applications in other countries (assignee: Hoffman La Roche, Inc).

**IMAC Sepharose products, Ni Sepharose products and Fe Sepharose products**  
These products are sold under a license from Sigma-Aldrich under patent number EP 1277616 (Metal chelating compositions) and equivalent patents and patent applications in other countries.

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