HisTrap HP and Ni Sepharose High Performance Media

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Ni Sepharose media and His Trap HP pre-packed columns utilize a new ligand chemistry to chelate metal ions for metal affinity chromatography. This media has a higher binding capacity and tolerates a wider range of chemical conditions than HiTrap MC media. HisTrap HP media and columns come pre-charged with Ni. It is recommended to strip and recharge the media/ columns between use with different targets. The column can be used up to 6 times with the same target.

Materials/Reagents/Equipment	**Vendor**
Reagents	
Distilled water	
Charging solution. 0.1 M $NiS0_4$.	Can also use $CuS0_4$, $ZnCl_2$, $NiS0_4$, $CoCl_2$ Fe2+, Ca2+, Co2+
Binding buffer: 50mM Hepes (pH 3 - 12), 0.3M NaCl, 10mM Imidazole	buffer A
Elution buffer: 50mM Hepes (pH 3 - 12), 0.1M NaCl, 1.0 M Imidazole	buffer B. Vendor recommends 0.5 M NaCl but can use from 0.1 to 1M NaCl
Stripping buffer: 50mM Hepes, pH 7, 0.3M NaCl, 0.05M EDTA.	
Equipment	
HisTrap HP pre-packed columns, 1 ×5ml, 17-5248-01: $107 5 x 1ml, 17-5247-01: $121	Amersham Pharmacia Biosciences Max. pressure = 43 psi, 0.3 MPa.

Column size	Protein binding	Max. Flow rate	Recommended flow rate/BSGC Load	Elute	Fraction Size
1 ml HisTrap	40 mg	4ml/min	1 ml/min	1 ml/min	1 ml
5 ml HisTrap	200 mg	20ml/min	2 ml/min	2 ml/min	3 ml

Chemical compatibility. The media is stable in most commonly used reducing, denaturing agents and detergents. Below is a list of tested substances and the concentrations tested.

DTT: 5 mM	Triton X-100: 2%	SDS: 1% for 1 hour
BME: 20 mM	Tween 20: 2%	
TCEP: 5 mM	NP-40: 2%	
urea: 8 M	cholate: 2%	
guanidine HCl: 6 M	CHAPS: 1%	

Procedure

A. Charging the matrix with metal ions
1. Remove the top-cap of the HisTrap Chelating column and apply a few drops of distilled water to the top of the column to avoid air bubbles.
2. Connect a dedicated water syringe containing 0.2u filtered deionized water to the HisTrap luer adaptor, let water drip out of it onto the top of the column and attach.
3. Remove the outlet cap.
4. With a dedicated charging syringe, load 0.5 CV 0.1 M $NiS0_4$ in distilled water on the column from top down. Collect any eluted fluids in a dedicated waste container. Remember, solutions with $NiS0_4$ are considered Hazardous waste and must be disposed of accordingly.
5. Fill syringe with distilled water, wash column with 5 CV distilled water, collecting eluate into a waste bottle.
6. Connect the column to AKTA, Biocad or other chromatography system. Equilibrate the column with 5CV of start buffer (50mM Hepes, pH 7, 0.1M NaCl, 10mM Imidazole). Recommended maximum flow rate: 5ml/min for 5 ml column or 1 ml/min for the 1 ml column.

Note: For a new column, start with step #5.

B. Binding is often strongest in the pH range of 7-8.
1. Sample preparation
The sample should be centrifuged and 0.45µ filtered.
2. Adjust the sample to 0.1M NaCl and 10 mM Imidazole, or desired concentrations.
3. Load the sample, using a pump at a flow rate of **2 ml/min for the 5 ml column**. Collect the flow-through in bulk (Erlenmeyer flask).
4. Wash the column with 5CV of start buffer or until no protein appears in the effluent. Excessive washing should be avoided since this may decrease the yield. Collect the Wash in bulk in an Erlenmeyer flask.

C. Elution
1. Elute with elution buffer of desired Imidazole strength (step gradient) at **2 ml/min for the 5 ml column** or a shallow gradient (can separate proteins with similar binding strengths). For **step gradient elution**, use 5 CV for each Imidazole strength. For **gradient elution**, use a selected number of CVs, i.e. 10 to 400 mM Imidazole in 13 CVs followed by 400 – 1000 mM imidazole in 3 CVs. Collect 3.0 ml for each fraction. Note: Since imidazole absorbs at 280nm, it may be hard to see the protein peak, so will need to do Bradfords to find where the protein is located. Also note that the protein may be in high salt and high imidazole, so if there is a need to load a large volume on SDS/PAGE, you may want to concentrate the sample using the Ultrafree units.

D. Stripping the column.
Strip the column of chelated metal ions after **each** experiment and re-charge it **before** re-use to achieve reproducible results and maintain selectivity and capacity.

1. Strip the column by washing with 5CV of 50mM Hepes, pH 7, 0.3M NaCl, 0.05M EDTA using a syringe. Collect this eluate in a hazardous waste container. It is recommended to strip the column in two steps. First, flush the 4 CV to waste. Second, let the final CV sit in the column for 5 minutes before flushing it out. This will give sufficient time for the full stripping of the metal ions to be accomplished.
2. Wash with at least 5CV of distilled water/0.05% azide using a syringe. Store at room temperature.

Alternatively, the column can be stored in 20% EtOH at 4C.