HIS-Tagged Bacterial Protein Purification Kit (Gravity Flow)

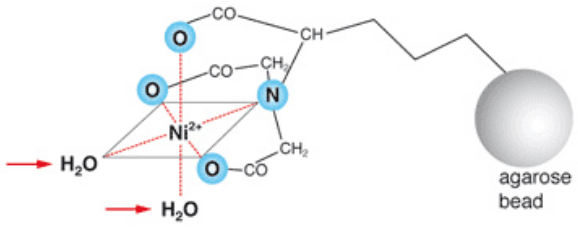
MBPP005-2NO

MBPP005-5NO

Introduction: Protein purification is an essential pre-requisite for proteomics. Protein purification procedures can vary from simple one-step precipitation procedures to large scale production processes. The most accepted of these methods is affinity purification where the protein of interest is purified through its specific binding properties to an immobilized ligand. Affinity tags are very efficient tools to purify heterologous proteins from different sources because the single step purification process involves mild elution conditions and do not interfere with the structure and function of the recombinant protein. For the purification of recombinant proteins additional amino acids or a whole protein is often added and is known as fusion tags. One of the most common fusion tag is 6xHis or polyHis tag (six histidine residues) which binds to nickel. Polyhistidine-tag is an amino acid motif in proteins that consists of at least six histidine (*His*) residues, often at the N- or C-terminus of the protein. Polyhistidine-tags are often used for affinity purification of polyhistidine-tagged recombinant proteins expressed in *Escherichia coli*. Affinity purification using a polyhistidine-tag usually results in relatively pure protein when the recombinant protein is expressed in prokaryotic organisms. Expressed His-tagged proteins can be purified and detected easily because the histidine residues bind metal ions like nickel immobilized on a column matrix under specific buffer conditions and bound proteins are eluted out by changing the pH or by adding a competitive molecule like imidazole.

Ni-NTA Agarose uses nitrilotriacetic acid (NTA), a tetradentate chelating ligand, in a highly cross-linked 6% agarose matrix. NTA binds Ni^{2+} ions by four coordination sites.

Structure of NTA in complex with Ni^{2+}



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HiMedia's HIS-Tagged Bacterial Protein Purification Kit (Gravity Flow) is used for rapid purification of recombinant proteins fused to 6X HIS and expressed in bacteria. The kit contains pre-packed ready-to-use columns of 2 ml Ni-NTA Agarose resin in 30% ethanol.

Binding capacity: 50 mg of HIS-tagged protein/ml gel

Kit Contents:

Product Code	Reagents	Quantity		Storage
		2 NO	5 NO	
DBCA14	Ni-NTA Agarose Column	2 Nos	5 Nos	2 - 8ºC
DS0113	10X Buffer	100 ml	4 X 125 ml	2 - 8ºC
DS0114	3M Imidazole	4 ml	20 ml	2 - 8ºC

Reagents required but not provided

- Molecular Biology Grade Water (Product Code: ML064)
- 0.5 M NaOH

General Preparation Instructions:

1. Dilute the 10X Buffer to 1X using Molecular Biology Grade water to prepare Equilibration Buffer, Wash Buffer and Elution Buffer.

2. Buffers may need some optimization depending upon the specific protein. The following table can be used to make buffers with different imidazole concentrations:

Table 1

Imidazole Final Conc. (mM)	10X Buffer (ml)	3M Imidazole (µl)	Water (ml)
10	1	33.3	8.97
25	1	83.3	8.92
40	1	133.3	8.87
60	1	200	8.80
75	1	250	8.75
150	1	500	8.50
200	1	667	8.33
250	1	834	8.17
500	1	1668	7.33

3. For most proteins the following imidazole concentration is recommended:

   Equilibration Buffer - 10mM imidazole in
   Wash Buffer - 25mM imidazole
   Elution Buffer - 250mM imidazole

Procedure for purification of HIS-Tagged Protein

1. **Elimination of the Preservative:** Remove first the upper cap and then the lower one of the column to allow elimination of the preservative by gravity flow.
2. **Equilibration of the Pre-packed column:** Equilibrate the column with 5X resin bed volume of Equilibration Buffer (prepared from 10X Buffer, Code DS0113 and 3M Imidazole, Code DS0114) and allow draining the buffer through column. Do not let the resin bed dry.
3. **Application of the Sample:** Close the bottom cap and add the sample containing the HIS-tagged protein to be purified (clarified *E. coli* lysate) through the top of the

column. Close the top cap and keep sample and resin in contact for at least 45 - 60 minutes before removing the bottom cap. Collect the flow through.

4. **Washing:** Close the bottom cap. Add 5X bed volumes of the Wash Buffer (prepared from 10X Buffer, Code DS0113 and 3M Imidazole, Code DS0114) through the top to eliminate all the proteins that have not been retained in the column. Close the top cap and mix manually inverting the column. Remove the bottom cap and discard the flow through. Repeat the step twice.

   Note: Wash the column until the absorbance at 280 nm of the eluent reaches the baseline.

5. **Elution of the pure protein:** Close the bottom cap and add the Elution Buffer (prepared from 10X Buffer, Code DS0113 and 3M Imidazole, Code DS0114) through the top. Close the top cap and mix manually inverting the column thoroughly for 15 minutes. Let the gel settled, remove the bottom cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice and pool the collected eluates.

   Note: It is possible that a significant amount of HIS-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among HIS-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

6. **Regeneration and Storage of Column:** During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary. Regeneration consists of the complete elimination of the retained protein.

   In general, column regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do regeneration when an appreciable reduction in the yield is observed. The frequency of these stages varies with the protein and the conditions used:

   I. Wash the resin with 0.5M NaOH for 30 minutes.

   II. Remove the NaOH by washing with 10 bed volumes of distilled water.

   III. Wash and resuspend with 30% Ethanol. Finally, store at 2 – 8ºC.

Columns can be regenerated for at least 5 times without significant loss in binding capacity.

Troubleshooting Guide:

Sr. No.	Problem	Cause	Solution
1.	High viscosity sample	Presence of DNA in the sample/lysate	Treat the lysate with DNase I
		Presence of insoluble material in the sample/lysate	Spin or filter to avoid clogging of column.
2.	Target protein does not bind to the column	His-tag has been degraded	Use protease inhibitors and do the purification at lower temperature.
		Tag may be inaccessible	Purify the protein in denaturing conditions or add the in other site.
		Inadequate binding conditions	Check the pH for binding
3.	High amount of protein contaminants	Insufficient washing	Increase the concentration of imidazole in the buffer during washing and equilibration steps (as mentioned in Table 1).
4.	Target protein elutes poorly	Protein binding with chelating metal is too strong.	If possible, try the elution procedure at higher temperature.
		Fused protein can be precipitated	Elute in denaturing conditions and incubate the column with elution buffer for a longer period of time.

Additional Information:

If the expressed proteins form inclusion bodies then the purification procedure has to be carried out in denaturing condition using 8M Urea or 6M Guanidine hydrochloride.

For denaturing conditions the following buffers can be used:

Denaturing Binding Buffer: 20mM Sodium phosphate, pH 7.8, 500 mM NaCl, 8M Urea

Denaturing Wash Buffer: 20mM Sodium phosphate, pH 6.0, 500 mM NaCl, 8M Urea

Denaturing Elution Buffer: 20mM Sodium phosphate, pH 4.0, 500 mM NaCl, 8M Urea

Safety Information

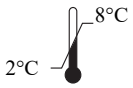
The HiMedia's HIS-Tagged bacterial Protein Purification Kit (Gravity Flow) is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. HIS-Tagged bacterial Protein Purification Kit (Gravity Flow) is designed and sold for research and in vitro purposes only. The product is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressed clearly for that purpose by the Food and Drug Administration or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in the text.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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