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HISTIDINE ADEM-KIT 04500

For His-Tagged protein purification

For research use only

INTRODUCTION

The kit is based on the use of the **Biomagnetic separation technology. The separation method is gentle and does not require the use of columns or centrifugation step.**

Biomagnetic separation technology is a simple technique based on the separation of **superparamagnetic beads** using a magnetic field. When added to a complex medium, the magnetic beads will bind to the target. This interaction is based on the specific affinity of the ligand on the surface of the beads. The resulting target-bead complex can be removed from the suspension using a magnet. The benefits of magnetic handling are easy washing, separation and concentration of the target without any need for centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

HISTIDINE ADEM KIT PRINCIPLE

Obtaining pure proteins is a critical step. To simplify the purification process, a successful approach has been to add "tags" to recombinant proteins during cloning and expression. One of the most commonly used tags consists of six to ten consecutive Histidine residues added to either the N- or C-terminus of the protein of interest. Tagged recombinant protein are exploited both to isolate protein complexes from crude cell extracts and to investigate protein/protein and protein/nucleic acid interaction.

Histidine Adem-kit provides a fast, efficient method for purifying 6xHis-tagged protein with high binding capacity and yield, and low background in a convenient flexible format.

The Ni magnetic beads are magnetic particles that have metal-chelating iminodiacetic acid (IDA) groups covalently bound to their surface. They are precharged with nickel. IDA has tridentate chelating group that occupies three of six sites in the nickel coordination sphere. The affinity of Histidine residues for immobilized nickel allows selective purification of Histidine-tagged protein. 6xHis-tagged proteins can be eluted from Ni magnetic beads with buffers containing imidazole.

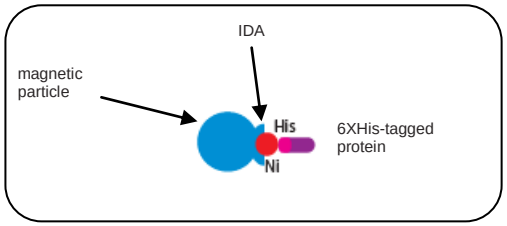


Figure 1: Interaction between NI-IDA magnetic beads and Histidine-tagged protein

The purified Histidine tagged recombinant protein can be used in a variety of applications including: structural and functional investigations, crystallization for determination of three-dimensional structure, assay involving protein-protein and protein-DNA interactions, immunization to produce antibodies.

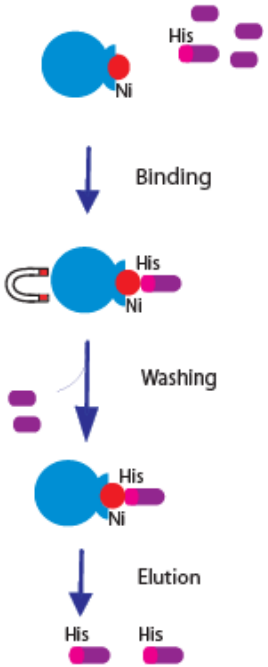
PRODUCT DESCRIPTION

The Kit contains all the components required for 65 Recombinant protein purification procedures.

	Amount	Component	Storage
R1	2 ml	**Magnetic beads**	+ 2-8° C
R2	180 ml	**Binding Buffer**	+ 2-8° C
R3	12 ml	**Elution Buffer 1**	+ 2-8° C
R4	12 ml	**Elution Buffer 2**	+ 2-8 °C

Table 1: Components provided with the kit

PROTOCOL SUMMARY



INSTRUCTIONS FOR USE

Optimal purification is dependant of the concentration of 6xHis-tagged protein in culture medium.

For best results we recommend adapting the binding step depending of the 6xHis-tagged protein concentration of starting material. For optimal performance, the binding capacity of the magnetic beads used in each purification should approximately match the amount of the 6xHis-tagged protein to be captured

However, it is up to the individual researcher to determine the suitability for their particular use.

A) Preparation of sample prior to purification

There are many different ways of preparing a cell lysate containing expressed Histidine tagged protein. The following is a general protocol for sample preparation.

1. Harvest cells from the culture by centrifugation at 6000 rpm for 5 minutes.

Lysis will be more efficient if cells were frozen at -20° C or -70°C.

2. Discard the supernatant. Place the cell pellet on ice.
3. Resuspend cells in 1 ml Binding Buffer.
4. Sonicate on ice to lyse cells (4 times for 30 s each time with 10 s pause between).
5. Clear lysate by centrifugation at 10000rpm for 15 min at 4°C.
6. Collect supernatant.

B) Binding step

For optimal performance, match the amount of Ni magnetic beads to the amount of the 6Xhis tagged protein to be captured.

The following protocol is established for 30µl Ni magnetic beads that have a binding capacity of 120µg 6xHis-tagged protein.

1) **Resuspend 30µl magnetic particles** in 300µl of Binding Buffer
2) **Place the tube on the magnet** until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add 300µl Binding Buffer and mix by vortexing or inverting the tube.
3) Repeat step 2.
4) Add 1 ml of the 6xHis-tagged protein sample.
5) Incubate the suspension and roll mix for 15 minutes at room temperature.

C) Washing step

1) **Place the tube on the magnet** until supernatant clearing and **discard the supernatant**. Remove the tube from the magnet and add 200µl of Binding buffer.

2) **Repeat 3 times** step 1 using **200µl Binding Buffer**.

D) Elution step

1) **Place the tube on the magnet** until pellet forming and **discard the supernatant**. Remove the tube from the magnet.

2) **Add 150µl Elution Buffer 1** and mix by vortexing or inverting the tube. After 1-5 minutes with gentle mixing at room temperature, place the tube on the magnet and **keep the supernatant** containing the 6xHis-tagged protein.

3) **Repeat step 2 using 150µl Elution Buffer 2. Keep the supernatant** containing **the second 6xHis-tagged protein Fraction.**

Elution volumes of less than 50 µl are not recommended because it is difficult to recover small volumes of elution Buffer.

ADDITIONAL MATERIAL REQUIRED

- Magnetic devices
  - Adem-Mag SV, 1.5 ml (# 20101)
  - Adem-Mag MODULO (# 20105)
  - Auto-Mag (# 20105)
- Microtubes
- Rotation device

TROUBLESHOOTING

Problem	Comment	Suggested
6xHis-tagged protein not bound to the magnetic beads	The binding step is not optimal	Increase binding time
	Sequence is not correct	Confirm the clone by sequencing
	Protein degradation	Add protease inhibitor to the lysis buffer
The yield is too low	Proteins not eluted from beads	Add one step of elution
	Difficult to elute protein	Elute with higher concentration of imidazole in the elution buffer
Isolated 6xHis-tagged protein is not pure enough	Non-specific binding caused by endogenous proteins	Use low concentration of imidazole (10 mM) in the binding/washing buffer
		Add one or two more washing steps, but note that the yield may decrease
		Dilute starting material
		Use lesser magnetic beads
Lysis step is incomplete	Viscous pellet, too much DNA	Add DNase to the lysate
	Not optimum lysis	Use lysozyme

APPENDIX

Composition of Buffers

Component	Composition
Binding Buffer	20mM Tris, 500 mM NaCl, pH 7.5, 0.09% sodium azide
Elution Buffer 1	20mM Tris, 500mM NaCl, 100mM Imidazole, pH 7.5 0.09% sodium azide
Elution Buffer 2	20mM Tris, 500mM NaCl, 500mM Imidazole, pH 7.5 0.09% sodium azide

Reagent Tolerance

Reagent	Effect
Histidine Binds to Ni-IDA	Competes with Histidine tagged protein
EDTA, EGTA Chelating agents	Remove Ni ions from the magnetic beads
β-mercaptoethanol **DTT, DTE** Prevent disulfide bond formation	-At high levels, may reduce the Ni irons -At low concentrations, reduce Ni ions
HEPES, MOPS Buffer with secondary or tertiary amines	At high levels, reduce Ni ions
Glycine Binds to Ni-IDA	Competes with Histidine tagged protein

STORAGE / STABILITY

Properly stored kits are guaranteed for 5 months from the date of receipt. Note that the shipping is realized at room temperature which will not affect the stability of the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination. If cytotoxic preservatives are added they must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For research use only. Not for use in human diagnostic or therapeutic procedures.

Sodium azide is toxic if ingested. **Avoid pipetting by mouth**. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide buildup.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.
Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

Ordering Information

Product	Description	Code
Histidine Adem-Kit	**65 Recombinant protein purifications**	**04500**

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