Amintra Nickel Magnetic

Data and Instructions

1. Introduction

His-tags have been widely employed as a powerful separation approach in the purification of a broad range of proteins and peptides. It is based on the specific interactions between certain transitional metal ions, mostly Cu2+, Ni2+, Zn2+ and Co2+ to the exposed amino acid surface chains containing histidine. The presence of several adjacent histidines such as (His)6-tag increases the affinity to immobilised metal ions. Increasingly, Amintra Ni-NTA and Ni-IDA resins are employed for the purification of histidine-tagged recombinant proteins expressed in bacteria, yeast and mammalian cells.

Amintra Nickel Magnetic resin is typically useful in purifying small quantity low concentration histidine-tagged proteins. For example, when 10 – 50 µl of resin is required for protein purification, it is difficult to handle such small quantities in columns. Amintra Nickel Magnetic resins can be easily processed with the aid of a magnet. The liquid / solid separation required in each purification step (e.g. equilibration, binding, washing and elution) is readily achieved with the aid of a magnet (e.g. magnetic stirring bar or other commercial magnetic devices).

2. Product characteristics

Amintra Nickel Magnetic is specially designed and fabricated for magnetic purification of proteins in batch mode (i.e. stirred tank mode). The base matrix is made of cross-linked polysaccharide encapsulating with fine magnetic particles. Therefore, the resin possesses magnetic property. Removal of liquid after each step such as binding, washing and elution can be readily done by fixing the resin with a magnet.

Note: no magnet is supplied with this product, so customers need to source the suitable magnet.

Table 1. Product characteristics

Particle size	50 – 150 µm
Base matrix	Cross-linked 6% agarose encapsulating magnetic particles
Metal ion capacity	Approx. 12 – 25 µmol / ml resin*
Protein binding capacity	Depends on the type of proteins and binding conditions; could be > 40 mg / ml resin*
Chemical stability**	Stable in 0.01M HCl and 1% SDS tested for 30 mins; 0.5 M NaOH tested for overnight
pH stability**	2-14 (<2 h) 4-12 (up to one week)
Storage	20% ethanol at 4°C

*Tested with nickel ion charged; **Tested in the absence of metal ions.

Amintra Nickel Magnetic is highly stable and compatible to a wide range of chemicals commonly experienced in protein purification processes (see Table 2), which means that more flexible operations can be developed for the best performance.

3. Purification procedures

Amintra Nickel Magnetic is specially designed for batch purification of small quantity of proteins. Target protein can be directly purified from unclarified or clarified cell lysates. Amintra Nickel Magnetic is compatible with most of the commonly used reagents in biological systems. Recombinant proteins expressed as inclusion bodies can be directly purified (and refolded if necessary) after dissolving in denaturing reagents such as 6 M GuHCl or 8 M urea.

3.1 Preparations before protein purification

Harvest the cells or broth after the culture is finished. Lyse the cells in order to release the proteins. Proteins expressed as inclusion bodies can be dissolved in denaturants such as GuHCl and urea. Clarified or unclarified protein samples can be purified directly. Unclarified lysates should be treated with DNAse I (e.g. 5 µg/ml of Basemuncher (benzonase) with 1 mM Mg2+ for 10-15 mins in ice-bath) to reduce the sample viscosity.

Cell lysis and addition of imidazole will change the pH. Precondition the lysates so the pH is suitable for purification. 0.5 M NaCl and or a low concentration of imidazole (e.g. 20 mM) can be added to the binding buffer to reduce non-specific binding. Note: Use the same concentration of imidazole and NaCl for lysate and the binding buffer.

Table 2: Compatibility of reagents with Amintra Nickel Magnetic*

Chelating reagents	EDTA, EGTA	Up to 1 mM, but care should be taken to any chelating reagents. It may be added to the samples rather than directly to the binding buffers.
Denaturing reagents	GuHCl Urea	Up to 6 M Up to 8 M
Detergents	Triton X-100 Tween-20 NP-40 CHAPS SDS	Up to 2% v/v Up to 2% v/v Up to 2% v/v Up to 1% Pre-testing required case to case, 0.1-0.3% might be ok
Reducing reagents	β-mercaptoethanol DTT DTE Reduced glutathione	Up to 20 mM Up to 2 mM Up to 2 mM Up to 10 mM
Buffer reagents	Sodium phosphate, pH 7.5 Tris-HCl, pH 7.5 Tris-acetate, pH 7.5 HEPES MOPS Sodium acetate, pH 4	Up to 50 mM, commonly recommended Up to 100 mM Up to 100 mM Up to 100 mM Up to 100 mM Up to 100 mM
Other additives	NaCl Ethanol Glycerol Imidazole Citrate Glycine Sodium biocarbonate Sodium sulphate	Up to 2 M, 0.5 M is recommended as a start point Up to 20% Up to 50 % Up to 500 mM Up to 60 mM Not suggested Not suggested Up to 100 mM

*Tested after Ni^{2+} ion is charged to the resin.

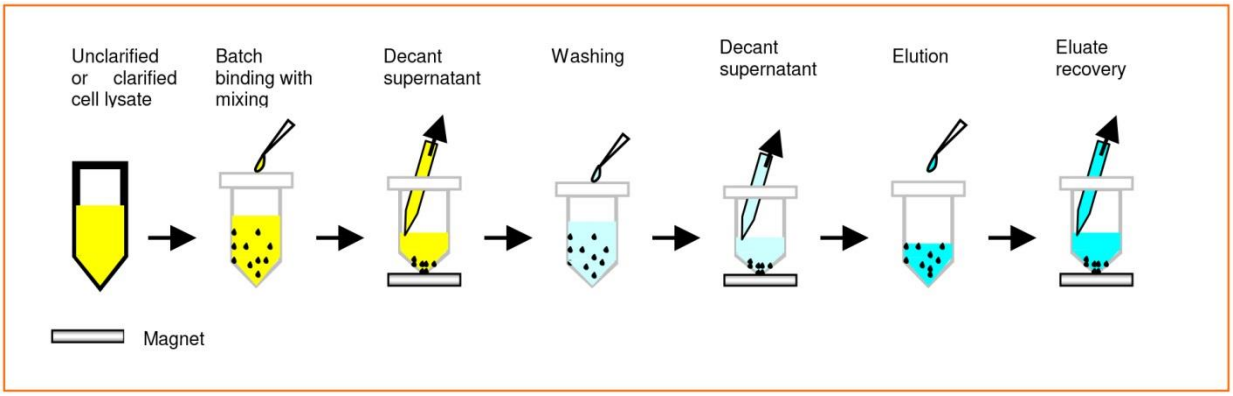
Recommended Buffers:

Equilibration / binding buffer: 20 mM sodium phosphate, 0.5 – 1.0 M NaCl, pH 7.4. Imidazole can be added to reduce non-specific binding at a concentration between 10 – 50 mM.

Washing buffer 20 mM sodium phosphate, 0.5 – 1.0 M NaCl, pH 7.4.. Additional reagents (e.g. detergents, alcohol and increased imidazole concentration etc) can be added to enhance removal of weakly bound impurities

Elution solution 20 mM phosphate buffer , 0.5 M NaCl, 250 mM – 500 mM of imidazole pH 7.4.

3.2 Protein purification procedure



1. Use 70 ul of resin slurry (35 ul of resin beads) for 1 ml of cell lysate contains 0.5 mg of his-tagged protein.
2. Shake the bottle to resupsend the resin beads. Take out the required amount of the slurry using a 200 µl pipette (Note: the pipette tip should be cut off approx. 3-5 mm from the narrow side to avoid possible tip blockage by the resin particles) and transfer to a suitable container (e.g. a eppendorf centrifuge tube) for batch binding.
3. Wash the resin with 5 - 10 resin volume of the equilibration / binding buffer. After the magnetic particles are settled by a magnet, remove the supernatant by a pipette. Repeat the washing two times more. Be sure the magnet is removed when washing buffer is added and mixed.
4. After washing the beads, add the protein sample. Close the container lid and place onto a suitable roller mixer for batch binding. Depending on the nature, size and concentration of the target protein the binding time can vary. Typically 10 – 30 minutes is sufficient to facilitate binding.
5. After the batch binding is finished, pull down the resin with a magnet. Remove the supernatant by a pipette. Use a strong magnet to avoid losing magnetic particles in the supernatant. Wash the resin three times (3 – 5 resin volume each time) with the washing buffer.
6. Elution can be done in batch. Use an eluent volume 3 – 5 times the resin volume to recover the bound protein. For the best recovery, incubate on a roller mixer for 5 - 10 minutes. Apply the magnet to pull down the beads and recover the protein in the supernatant. This step may be repeated once or twice to maximise recovery.
7. Buffer exchange and / or desalting may be required to adjust to remove imidazole and salt from the eluted sample. Depending on the sample volume, Amintra Desalting Spin columns can be used for fast and cost-effective desalting /buffer exchange.

4. Trouble shooting

No target protein in the eluted fractions	1. **Elution conditions are too** mild to dissociate the bound protein. Increase the imidazole concentration or further reduce the pH in the elution buffer. If hydrophobic interaction is contributing, addition of non-ionic detergents (e.g. Tween-20) can improve the recovery. Elute with EDTA if the problem persists. 2. Incorrect binding conditions. Check pH and composition of all buffers. The concentration of imidazole in the binding buffer may be too high. 3. **Histag is not present.** Check the construct. Perform a western blot with anti-histag antibodies 4. **Histtag is not accessible**. use denaturing reagents such as urea to partially unfold the protein. 5. The target protein has precipitated on the resin: Use NVoy to enhance protein solubility.
The target protein is eluted with impurities	1. Increase the imidazole concentration in the binding and washing buffers. 2. **Impurities are associated with the target protein.** Add reducing reagents (e.g. <20 mM β-mercaptoethanol) in the sample or washing buffer to disrupt formation of disulfide bond. Add NVoy, detergents or glycerol in the washing buffer to suppress any non-specific interaction. 3. **Impurities are truncated parts of the target protein.** Check the gene construction and expression conditions to minimise potential mutations. Prevent protein degradation by addition of protease inhibitors work at 4C

5. Storage

Store the resin in 20% ethanol at 4oC. Seal the bottle lid after each use.

6. Further information

Visit www.expedeon.com for further information or contact the technical team or a sale representative.

8. Ordering information

Product	Unit Size		Code no.
	Medium (ml)	Slurry (ml)	
Amintra Nickel Magnetic	1	2	AMN0001
Amintra Nickel Magnetic	2.5	5	AMN0005
Amintra Nickel Magnetic	12.5	25	AMN0025

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