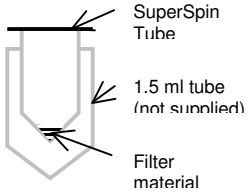
BIOTOOLOMICS

Ni SuperSpin \ Co SuperSpin \ Zn SuperSpin

Data and Instructions

IMAC SuperSpin is supplied as a low cost, single use disposable device that allows rapid purification and screening of histidine-tagged proteins at much less cost. The spin tube is filled with novel metal immobilised chromatography resin of small particles (20 – 50 μm). It gives very fast mass transfer rate of target proteins. So large amounts of protein can be captured in a short solid / liquid contact time. Three types of spin tubes are available as Ni SuperSpin, Co SuperSpin and Zn SuperSpin for fast screening of the best immobilised metal ion to a given target protein. It is a particularly powerful tool in applications such as small-scale purification, high-throughput screening, purification process optimisation etc.

Configuration of SuperSpin Tube



Each SuperSpin tube is packed with 50 μl resin.

The key benefits of IMAC SuperSpin

- Fast purification of tens of protein samples with a standard microcentrifuge.
- High protein binding capacity, as very small IMAC particle with slow settling rate is employed to give fast mass transfer.
- Very low cost in comparison to similar product in the marketplace.
- Optimisation of the binding, washing and elution conditions (e.g. imidazole concentration, pH, salt concentration, detergents etc) in one or two sets of experiments at short period and very low cost.
- Direct purification of protein from clarified and possibly unclarified sample.

The IMAC resin supplied has the characteristics listed in Table 1. It is very stable and compatible to a variety of chemical reagents as listed in Table 2. The leakage of metal ion is negligible.

Table 1. Characteristics of Ni SuperSpin / Co SuperSpin / Zn SuperSpin

Tube material	Polypropylene tube body with polyethylene frit
Medium	IMAC SuperSpin
Particle size	20 – 50 μm
Packed volume	50 μl
Protein binding capacity	Depends on the type of proteins and binding conditions; could be over 800 μg*
Chemical compatibility	Stable in the commonly used buffers and denaturing reagents, avoid chelating reagents e.g. EDTA, EGTA and citrate, see Table 2 for details
Storage condition	4°C – 8°C

*Tested with nickel ion charged

Table 2. Chemical compatibility*

Chelating reagents	EDTA, EGTA	Up to 1 mM, but care should be taken with 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	Up to 50 mM, commonly recommended Up to 100 mM Up to 100 mM

	HEPES	Up to 100 mM
	MOPS	Up to 100 mM
	Sodium acetate, pH 4	Up to 100 mM
Other additives	NaCl	Up to 2 M, 0.5 M is recommended as a start point
	Ethanol	Up to 20%
	Glycerol	Up to 50 %
	Imidazole	Up to 500 mM
	Citrate	Up to 60 mM
	Glycine	Not suggested
	Sodium biocarbonate	Not suggested
	Sodium sulphate	Up to 100 mM

*Tested with Ni^{2+} being charged

Preparations before protein purification

Protein expression and cell culturing are referred to in the well-established protocols in the literature.

Harvest the cells and / or broth after the culture is finished. For intracellularly expressed proteins, the pelleted cells are generally resuspended in PBS, tris-HCl buffer or other suitable buffers for following cell disruption such as freeze-thaw, ultrasonication, homogenisation and bead milling etc. Or the pelleted cells can be directly suspended into a self-made or commercial cell lysing solution for releasing of the target protein (refer to the well established protocols in literatures). Proteins expressed as inclusion bodies can be dissolved in denaturing reagents. 8 M urea is recommended.

Remove the cell debris by centrifugation at the top speed for 5 mins if a clarified sample is loaded. If an unclarified sample is loaded, it is recommended to conduct enzymatic lysis with lysozymes (200 μg/ml, incubation at 37°C for 30 mins) followed by a mechanical lysis e.g. sonication. If the sample is very viscous, a treatment with DNAse I (e.g. 5 μg/ml of Benzonase with 1 mM Mg^{2+} for 10-15 mins in ice-bath) may be required to reduce the sample viscosity. Pre-conditioning of the cell lysates, such as addition of 0.5M NaCl and low concentration of imidazole (e.g. 20 mM), pH adjustment etc, can be done in this step. ***Note***: imidazole and NaCl of the same final concentrations should be added to the lysate and the binding buffer; cell lysis and addition of imidazole will change the sample pH so adjustment of pH before sample loading is essential.

Equilibration / binding buffer is recommended as: 20 mM sodium phosphate + 0.5 M NaCl, pH 7.4. For the purification of his-tagged proteins, the presence of low concentration of imidazole is recommended. The exact concentration is protein and metal ion dependent with a guided range of 10 – 50 mM. See ***General considerations and optimisations*** for more information.

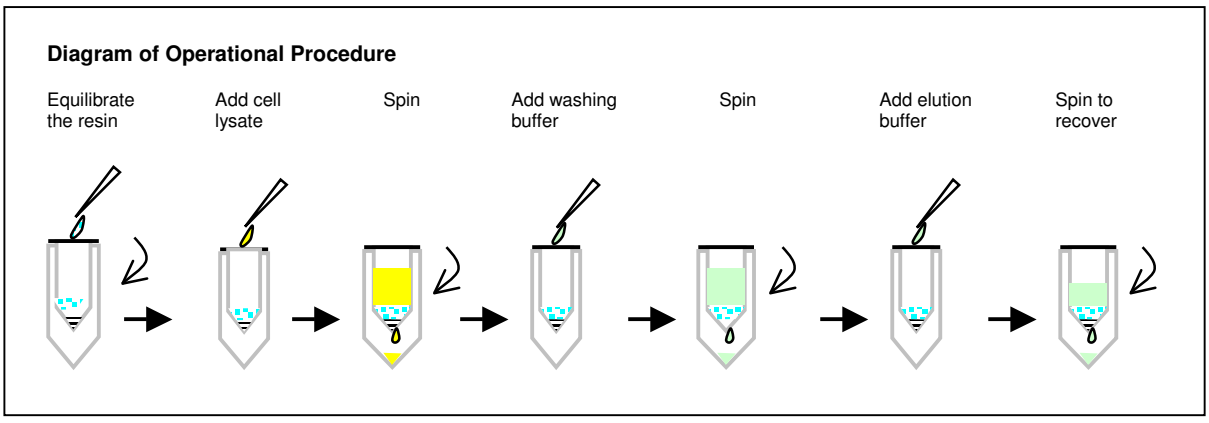
Washing buffer may contain additional reagents (e.g. detergents, alcohol and increased imidazole concentration etc) or have low pH value etc, in order to remove as much weakly bound impurities as possible. Refer to ***General considerations and optimisations*** for more information.

Elution solution should be prepared according to the guidance set in ***General considerations and optimisations***. The standard one can be 250 mM – 500 mM of imidazole in 20 mM phosphate buffer containing 0.5 M NaCl, pH 7.4.

Water and chemicals used for the protein purification process should be of high purity.

Purification operation

Conduct the purification in a standard benchtop microcentrifuge. Standard 1.5 ml microcentrifuge tube is required to collect liquid. The operation can be done at room temperature or at 4°C.



1. Invert and shake the SuperSpin tube to fully re-slurry the resin.
2. Snap off the bottom closure and loosen the cap. Place each SuperSpin tube in a 1.5 ml centrifuge tube. The lid of the 1.5 ml tube can be cut off if it interferes the spin process.
3. Spin at 1000 rpm for 30 seconds to settle down the particles. Empty the 1.5 ml tube.

4. Unscrew the cap of the SuperSpin tube, load 250 μl of the equilibration / binding buffer and spin at 1000 rpm for 30 seconds.

5. Take out the SuperSpin tube and fully empty the 1.5 ml centrifuge tube. Add upto 500 μl of the protein sample in. Screw the cap and invert the SuperSpin tube a few times to fully re-suspend the resin. Spin at 1000 rpm for 1 minute. (*Note: depending on the nature and conditions of individual protein lysate samples, spin at higher speed or longer time might be required to fully remove the liquid. The maximum spin speed is 6,500 rpm in a standard microcentrifuge*). More volume of sample can be added after each spin as long as it doesn't exceed the binding capacity. Make sure to fully empty the 1.5 ml tube after each spin. Typically, the protein sample is loaded 2 – 3 times (e.g. 1ml to 1.5 ml).

6. Fully empty the 1.5 ml tube or use a new one. Add 250 μl of the washing buffer and spin at 1000 rpm for 30 seconds (or longer time or higher speed if it is required in Step 4). This washing step is repeated at least one more time (with increased washing stringency if higher purity is required. Refer to ***General consideration and optimisations*** for further information). ***Make sure to fully empty the 1.5 ml tube after each spin.***

7. Place the SuperSpin tube in a fresh 1.5 ml tube. Add 150 μl of the elution buffer. Shake to fully re-suspend the resin. Spin at 1000 rpm for 1 minute. Collect the eluted liquid. Place the SuperSpin tube in another fresh 1.5 ml tube. Add another 150 μl of the elution buffer and spin at 1000 rpm for 1 minute. The liquid after two elutions can be collected into two separate 1.5 ml tubes or into the same tube. Most of the protein will be eluted in the first fraction.

8. Desalting or buffer exchange might be required to remove imidazole and salt in the eluted sample. SuperSpin Desaltor (product code: 210101) can be used for fast and cost-effective desalting /buffer exchange. Refer to the product files (www.biotoolomics.com/product) for more details.

General considerations and optimisations

1. Choice of immobilised metal ions

IMAC SuperSpin is supplied as three different types corresponding to the three most commonly immobilised metal ions, i.e. Ni^{2+}, Co^{2+} and Zn^{2+}, respectively. The choice of metal ions mainly depends upon the nature of target proteins and the specific application requirements. Ni^{2+} is commonly the first choice for purification of histidine-tagged recombinant proteins. As the strength and selectivity of interaction between a target protein and immobilised metal ion is affected by a few factors including the length and exposed position of the tag, electron distributions of the pair, pH and competitions from other impurities etc, some tagged proteins might be better purified with Zn^{2+}or Co^{2+} rather than Ni^{2+}. The similar considerations apply to purification of untagged proteins as well. A screening of different charged metal ions in combination with the specific application requirement (e.g. purity or yield or both) and binding conditions is recommended.

2. Choice of operational mode

Centrifugal (spin) column operation normally allows rapid and high throughput purification on a small scale. The penalty could be the less utilisation of resin capacity as the contact time is shorter. To address the above problem, IMAC SuperSpin with much smaller particle size (i.e. 20 – 50 μm) is used. In addition, the spin tube has been designed to extend the contact time between resin and protein sample. The above spin tubes are carefully designed to allow faster purification but with longer contact time between resin and protein sample. The overall purification cost by using IMAC SuperSpin is very low. It is a particularly efficient and cost-effective tool in optimisation of the purification conditions before committing to larger scale purifications.

Depending on the application requirements, there are also other operational modes for consideration, i.e. batch stirred tank mode (including magnetic operation with magnetic particles), gravity flow mode and pressurised (packed) column mode.

For laboratories that have no access to expensive and sophisticated process chromatography systems (e.g. packing columns, pumps and valves, detectors and associated computing machinery etc), or to whom protein purification is only a tool rather than their core research activities, the best choice is among batch stirred tank mode, gravity flow mode and centrifugal (spin) mode.

Batch stirred tank operation is the most versatile and flexible approach. Very little training in chromatography is required. It is particularly attractive in cases when the volume of protein sample is large but the concentration is relatively low or the target protein molecule is large (e.g. over 65K Dalton). Longer contact time can be employed in this mode to allow protein molecules diffusing into the pores, which means better utilisation of the whole resin ligands and subsequently higher binding capacity. Also, there is no limitation from the column side. So, large volumes of protein sample can be processed in the same time period as that for small volumes. IMAC SepFast resin is particularly suitable for this operation. As special columns (BG-30 for working volume of up to 30 ml; BG-5 for working volume upto 5 ml) is supplied to facilitate the separation between liquid and the solid resin particles, the hassle associated with the conventional batch operation, such as slow gravity settling of resin, centrifugal settling of resin and decanting of liquid (with potential loss of resin), have been removed.

Magnetic IMAC resin such as IMAC SepFast Mag is very suitable for batch stirred tank operation, particularly when the volume of protein sample is small (e.g. up to a few mls). Solid / liquid separation in each step (e.g. equilibration, binding, washing and elution etc) is readily achieved by a magnet.

Gravity flow operation is a good choice if the volume of protein sample is from a few mls to tens of mls and the protein molecule isn't very big. More than one column can be easily operated in parallel. IMAC SepFast resins possess strong mechanical structure to permit liquid to pass through at an excellent flowrate. The particle size and pore structure are carefully controlled for such operations. The specially designed columns (BG-30 and BG-5) are ideal for such operations with great flexibility.

Pressurised (packed) column operation gives the best chromatographic performance. IMAC SepFast PC is particularly designed for such operations. However, expensive and specialised instrument and accessories are required for such kind of operations. Professional training and expertise in process chromatography is essential in order to gain the most benefit from such operations. Unclarified cell lysates can't be directly processed in packed column, as cell debris will severely block the column.

3. Binding conditions

Proteins tagged with one or more 6 x His in either the N-terminus or C-terminus can be strongly bound to the metal charged IMAC SuperSpin resin. The interaction doesn't depend on the three-dimensional structure of the protein, as long as two or more than two chelating residuals in the protein can access the immobilised metal ion to form chelating bonds. Host cell proteins (HCP) that contain histidine, cysteine or tryptophan in a close proximity might interact with the resin but the strength is generally much weaker.

Three key factors are commonly optimised for the best binding performance. They are imidazole, pH and salt. The addition of imidazole of low concentration to the protein feedstock can effectively compete off the HCP binding caused by their chelating residues. Imidazole is usually added to the sample, equilibration buffer and binding buffer at a final concentration of 10 – 50 mM. The exact concentration of imidazole has to be optimised to balance two key parameters (i.e. purity and yield). Sodium chloride must be added to the sample and binding buffer to suppress any non-specific interaction caused by electrostatic charges. Commonly 0.5M of NaCl is used but further optimisation might be required to improve the product purity. In some cases, manipulation of pH (particularly reducing pH value in the sample) can improve product purity, as the chelating residues in HCP can't form chelating bond after they are charged.

Phosphate buffer is recommended in most cases. Tris-HCl buffer at lower concentration (e.g. 10-50 mM) is normally fine but care should be taken if a higher concentration is employed, as it might affect the binding in the case that the affinity of target protein to the metal ion isn't very strong. Addition of other chelating reagents such as EDTA at a very low concentration (e.g. < 1 mM) might improve the product purity in some cases.

Proteins expressed as inclusion bodies can be purified after dissolved with 6 M GuHCl or 8 M urea.

The amount of resin used to purify a given amount of target protein can be considered as well. The addition of much excess amount of resin might promote undesired non-specific binding of HCP as more free ligands are available to impurities.

4. Washing conditions

Stepwise increase of the washing stringency is recommended for the batch or gravity flow operations. In some cases, longer contact time may help to dissociate the bound impurities from the resin. Increased imidazole concentration, increased salt concentration, reduced pH, addition of denaturing reagents like GuHCl and urea, addition of alcohol (e.g. 30%) or glycerol (10 – 50%), addition of detergents like Triton and Tween etc are the commonly used approaches to remove the weakly bound impurities. Reducing reagents can be added in the washing buffer if disulfide bonds between HCP and the target protein may have been formed. A compromise between the final yield and product purity should be considered to develop the best washing conditions.

5. Elution conditions

There are mainly three choices of elution approaches: pH, imidazole and EDTA.

When the pH is reduced from neutral to less than 6 (typically 4 to 5.5), the histidine residues in the bound protein are protonated. Under this condition, the chelating bond between the metal ion and the histidine residues is dissociated. As a result, the bound protein is released. Care should be taken to investigate the stability of the target protein under a low pH value like 45.

Imidazole at high concentration (e.g. 100 – 500 mM) can effectively compete off the bound protein as well. The best elution concentration has to be determined case by case. This is the mildest approach among the three mentioned here.

EDTA is a very strong chelating reagent. 100 mM EDTA can fully stripe off the immobilised metal ion and therefore release the bound protein as well. It might not be desirable for metal ion being present in the final protein product.

Trouble shooting

The following tips may help to resolve the possible problems with individual purification process. If you would like further assistance, please contact our technical team or sales representatives for more information.

Low protein yield in the eluted fractions	1. **Elution condition is too mild to dissociate the bound protein.** Increasing the imidazole concentration or further reducing the pH in the elution buffer may help. If hydrophobic interaction is contributory, addition of non-ionic detergents (e.g. Tween-20) could improve the recovery yield. Elution with EDTA might be a choice in some cases. 2. **Binding conditions are not correct**. Check pH and composition of all buffers and solutions in each step. It should be pointed out that the addition of some reagents (e.g. imidazole) could cause the change of pH value. The concentration of imidazole in the binding buffer might be too high. 3. **Histidine-tag has been degraded**. Use anti-his antibodies in western blotting to check the location of the tag. 4. **Histidine tag is not sufficiently accessible**. Denaturing reagents such as urea could be added to partially defold the protein. 5. **The target protein has precipitate on the resin**. Try to add detergents (e.g. 0.1 – 1 % v/v) or denaturing reagents (e.g. 4 – 8 M urea). The concentration of NaCl might be reduced. Loading of less amount of protein and reduction of adsorption time can help to minimise such problems. 6.
The target protein is eluted with impurities	1. **Binding and washing conditions not stringent enough**. Refer to the Section *General considerations and optimisations* for further consideration. 2. **Impurities are associated with the target protein**. Try to add reducing reagents (e.g. <20 mM β-mercaptoethanol) in the sample or washing buffer to disrupt formation of disulfide bond. Try to add detergents or alcohol / 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 inhibitor and / or reduction of working temperature. 4. **Impurities have higher affinity than the target protein**. Careful optimisation of the binding, washing and elution conditions might allow the recovery of target protein without co-elution of the tightly bound impurities. 5. **Change of metal ion of choice**. IMAC SuperSpin charged with other metal ions might help. A choice of three types is available as: Ni SuperSpin, Co SuperSpin and Zn SuperSpin.
Liquid not fully removed in each spin	1. **The unclarified protein sample is too viscous**. Treatment by further mechanical disruption like ultrasonication or addition of DNase (e.g. 5 μg / ml Benzonase + 1 mg / ml of Mg^{2+} in ice-bath for 10-15 mins) can reduce the viscosity. Dilution with the binding buffer can help as well. Longer spin time can be tried. Use a clarified sample instead of an unclarified one. 2. **Protein severely precipitates**. Add detergents, denaturing reagents or other additives (refer to Table 2 for compatibility) to improve the protein solubility.

Further information

Visit www.biotoolomics.com for further information or contact the technical team or sales representatives.

Ordering information

Product	Quantity	Code no.
Ni SuperSpin	50	150101
Co SuperSpin	50	150103
Zn SuperSpin	50	150104
Ready-to-Use His Buffer Kit	2 x 50 ml phosphate stock solution (0.16 M sodium phosphate + 4 M NaCl, pH 7.4) and 50 ml imidazole stock solution (2 M, pH7.4)	200105

Related products

SuperSpin Desaltor	50	210101

Other IMAC products	Quantity	Code no.
IMAC SepFast	10 ml	180112
	25 ml	180101

	100 ml	180102
Screening kit of IMAC SepFast BG	5 ml each of Ni SepFast, Co SepFast and Zn SepFast, respectively; plus 1 Ready-to-Use His Buffer kit	180103
Ni SepFast	10 ml	180113
	25 ml	180104
	100 ml	180105
Pre-packed Ni SepFast	1 x 1 ml	180114
	1 x 5 ml	180116
Co SepFast	25 ml	180108
	100 ml	180109
Zn SepFast	25 ml	180110
	100 ml	180111

Magnetic Resin	**Quantity**	**Code no.**
Ni SepFast MAG	5 ml	190101
Co SepFast MAG	5 ml	190103
Zn SepFast MAG	5 ml	190104

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