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Immobilized metal ion affinity chromatography (IMAC)

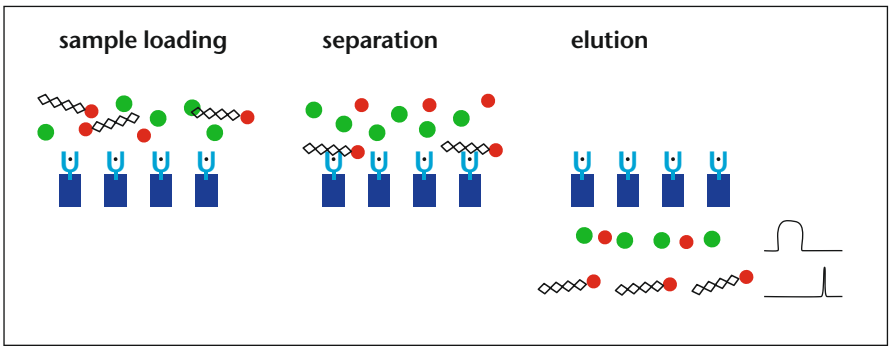
purification of recombinant antibodies

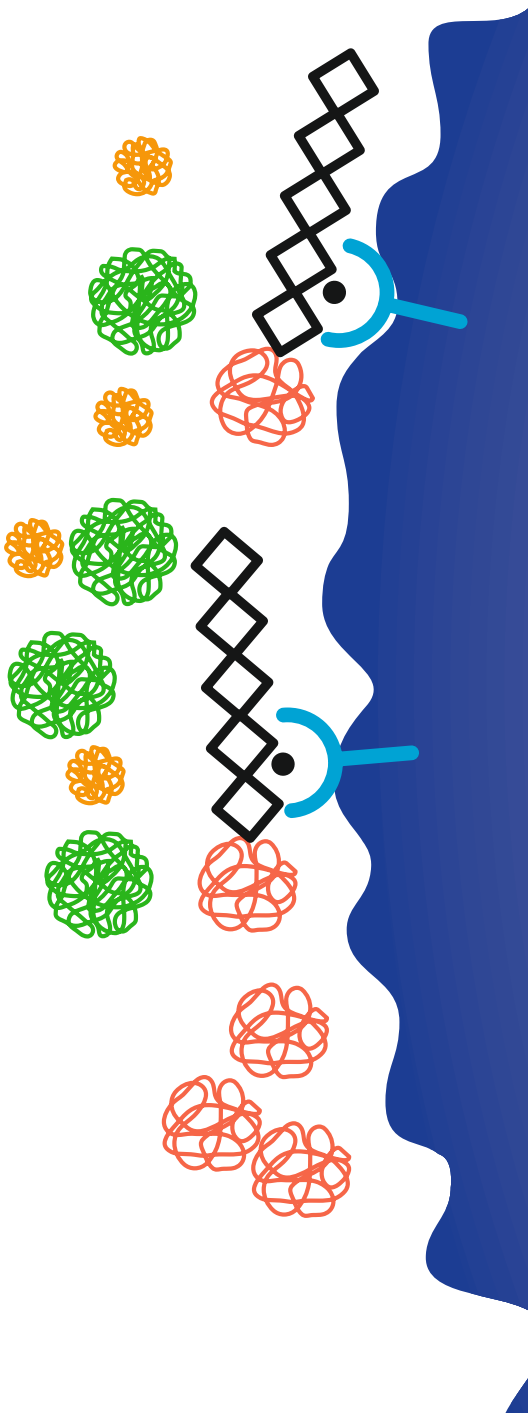
one step purification of His-tagged proteins

▶ BioFox 40 IDAhigh/low and TRENhigh/low

High throughput agarose-based media

Immobilized metal ion affinity chromatography is based on a high affinity binding of an immobilized metal ion by chelating a part of the target protein. Performed on a preparative chromatographic medium, IMAC is a highly efficient procedure to purify histidine-tagged proteins from a cell extract in just one step.
Typical metal ions such as nickel and cobalt selectively retain histidine-tagged proteins, but recombinant antibodies can also be purified by IMAC. In general, IMAC purification is the preferred technique when high yields of pure and active protein are required.





Most frequently used metal ions for the purification are Zn^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{3+}. Ni^{2+} and Co^{2+} ions are commonly used for histidine-tagged proteins whereas Fe^{2+} and Ca^{2+} ions are used for unknown binding characteristics of a target protein. Co^{2+} and Zn^{2+} ions strongly bind untagged proteins as well as histidine-tagged proteins.

Pressure stability up to 40 bar (580 psi) – fast and high resolution biochromatography

BioFox 40 IMAC media for immobilized metal ion chromatography (IMAC) are manufactured from agarose beads using a proprietary cross-linking method that results in a highly porous and physically stable agarose matrix. Besides the well-known selectivity of agarose, these media are pressure resistant up to 40 bar (580 psi) for high throughput biochromatography.

Two chelators are available for preparative scale IMAC: BioFox 40 IDA and BioFox 40 TREN.

Because sometimes the ligand density can have a great impact on the separation, BioFox 40 IDA and BioFox 40 TREN media are available with low and high metal ion loading capacities to allow for maximum flexibility when selecting the optimum IMAC conditions.
All BioFox 40 IMAC media generate sharp peaks with loadabilities of larger than 100 mg/g.

BioFox 40 IDA

- Made from agarose, well-established and well-known in the biotechnology industry
- Choice of IMAC chemistry to selectively bind a large range of proteins
- High flow characteristics
- Strong binding

BioFox 40 TREN

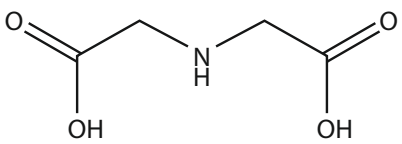
- Made from agarose, well-established and well-known in the biotechnology industry
- Choice of IMAC chemistry to selectively bind a large range of proteins
- High flow characteristics
- Weak binding

Therefore, the chelating efficiency is improved due to better access of proteins to the chelating sites.

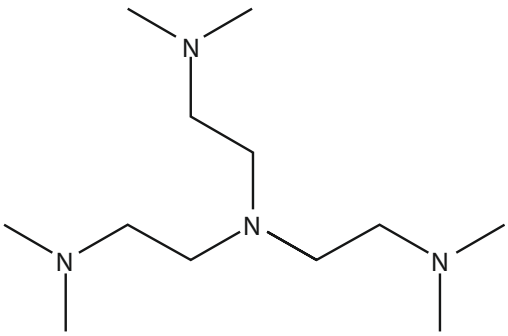
Because TREN shows a weaker binding, the non-specific binding of other proteins with intrinsic histidine groups together with the target protein is reduced. Therefore, optimization work to decrease non-specific binding is lesser than compared with strong binding IDA chelating groups.

Chelating groups

Iminodiacetic acid (IDA)



Tris(2-ethylaminoethyl) amine (TREN)



For further reading, we recommended the following article: "How to use immobilized metal ion affinity chromatography", A companion to Methods in Enzymology 4, 4-134 (1992) by Joy J. Winzerling et al.

Tips for accurate IMAC operation

Selected metal ions

Most frequently used metal ions for the purification with IMAC are Zn^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{2+} , but principally all metal ions known to interact with proteins can be used. For BioFox 40 IMAC media,the metal ion capacity is only specified for copper and will vary slightly for other metal ions.

Metal ion loading

A 50 mM solution of a selected metal ion is prepared in distilled water. Some care has to be taken when selecting the loading buffer. The metal ion concentration will be rather high when adsorbed to the gel and precipitation may occur.

Normally, a 0.1 M sodium acetate buffer at pH 5.5 can be used. The metal salt solution is loaded via a sample loop by repeated injections into the column until the media is fully loaded.

Removal of metal ions

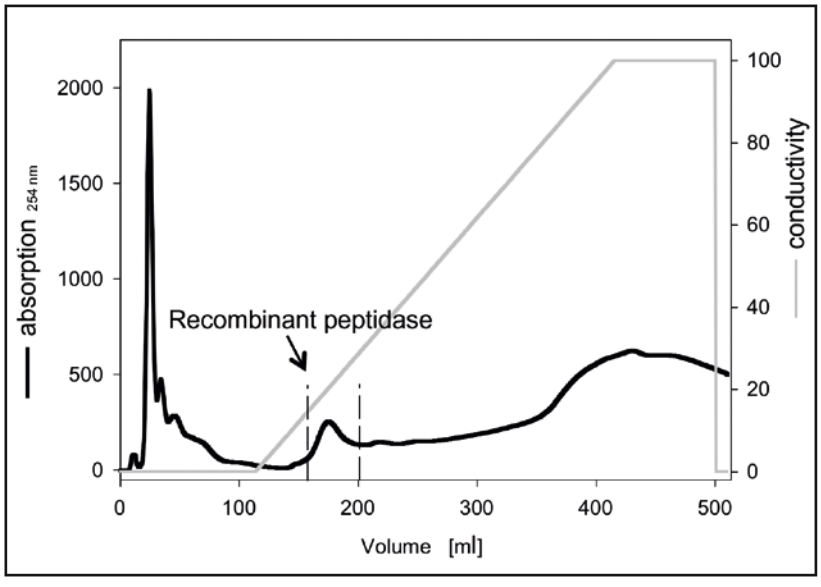
Many metal ions undergo redox reactions and this may cause deviations during storage of the media. If the gel is not going to be used for a long period, it is recommended to remove the metal ions from the column bed. This is easily done with 0.1 M of ethylendiaminetetraacetic acid (EDTA) solution, either through repeated injections via sample loop or by directly pumping the eluent through the column.

According to the separation problem, these operating conditions are vary. For adsorption, normally aqueous solvents could be applied, but organic solvents in low concentrations can also be used. Depending on the chelator's nature, both electrostatic and hydrophobic ineractions may be involved in the chelating complex formation. Additional care must also be taken regarding the ionic strength of the buffer solution. Buffers containing competitive groups with affinity for metal ions, such as imidazole, should be avoided.

Competitive elution with ammonium salts or imidazole buffer as well as decreasing pH value will elute the bound protein.

Applications

Because not all proteins show the same behaviour, four different IMAC chemistries are available. It is recommended to start with IDAhigh as this medium has the highest capacity and works well for most of the proteins. If the proteins are difficult to desorb from the column or elute with less activity, this reveals too strong binding. In this case, please try IDAlow. If this medium also fails, then try TRENhigh followed by TRENlow. The sequence will allow you to determine for the best separation medium for your application.



Purification of a recombinant peptidase using preparative BioFox 40 TRENhigh

Cleaning a recombinant peptidase (His-Tag®) from *E.coli* using BioFox 40 µ TRENhigh (loaded with $NiSO_4$)

The Bioline Rack – with glass columns

Media characteristics	BioFox 40 IDA	BioFox 40 TREN
Chelating group	Iminodiacetic acid (IDA)	Tris(2-ethylaminoethyl)amine (TREN)
Metal ion capacity	10 – 20 IDAlow	10 – 20 TRENlow
µeqv [Cu^{2+}/ml]	40 – 50 IDAhigh	50 – 60 TRENhigh
Max flow rate at 20 cm bed height [cm/h]	> 500	
Particle size [µm]	32 – 60	
Agarose content [%]	7.4 – 7.8	
pH stability	2 – 14	
Solvent stability after coupling the ligand	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid	

Available sizes (pre-packed columns)	BioFox 40 IDA	BioFox 40 TREN
	IMAC agarose media loaded with Cobalt or Nickel – pre-packed in analytical grade columns – preserved with 22 % ethanol	
Media volume [ml]	2.5	
Internal diameter [mm]	8	
Bed height [mm]	50	

BioFox 40 IDA and BioFox 40 TREN media are supplied in aqueous suspensions with 22 % ethanol as preservative and are immediately ready for use after washing.

Ordering information (Bulk material)	
Y4034	BioFox 40 IDAhigh, 25 ml
Y4024	BioFox 40 IDAhigh, 150 ml
Y4094	BioFox 40 IDAhigh, 1 l
Y4035	BioFox 40 IDAlow, 25 ml
Y4025	BioFox 40 IDAlow, 150 ml
Y4095	BioFox 40 IDAlow, 1 l

Ordering information (Bulk material)	
Y4014	BioFox 40 TRENhigh, 25 ml
Y4004	BioFox 40 TRENhigh, 150 ml
Y4084	BioFox 40 TRENhigh, 1 l
Y4015	BioFox 40 TRENlow, 25 ml
Y4005	BioFox 40 TRENlow, 150 ml
Y4085	BioFox 40 TRENlow, 1 l

Ordering information (pre-packed columns)	
Y4004NPG	BioFox 40 TRENhigh-Co 2.5 ml
Y4004NPN	BioFox 40 TRENhigh-Ni 2.5 ml

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