WorkBeads™40 IDA^{high}
WorkBeads™40 IDA$_{low}$
WorkBeads™40 TREN^{high}
WorkBeads™40 TREN$_{low}$

High Throughput Agarose Media for Immobilised Metal Affinity Chromatography (IMAC)

- **Made from agarose, well established and well-known in the biotechnology industry**
- **Choice of IMAC Chemistry to fit a large variety of proteins**
- **High flow characteristics**

Media Desescription

WorkBeads™40 IMAC media are produced from agarose using a proprietary cross linking method that results in a highly porous and physically stable agarose matrix. Agarose based matrices have been successfully used for decades in biotechnology research and in the industrial purification of proteins. Agarose is proven to be exceptionally compatible with natural bio-molecules like proteins, DNA, carbohydrates etc. The material shows minimal non specific interaction due to the hydrophilic nature of agarose. Unlike matrices made from synthetic polymers, agarose does not have micro pores that can contribute to local pH variations in the micro-environment in the column which lead to distorted separations.

WorkBeads™40 IDA and WorkBeads™40 TREN series of immobilised metal affinity chromatography (IMAC) gels are activated and coupled according to the Bromohydrin method. This method gives rise to a spacer arm between the agarose backbone and the attached chelator.

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

Ligand density has been shown to have a great impact on the separation. In some case a high capacity gel is needed while in other cases a low capacity gel may solve the problem. Therefore, **WorkBeads™40 IDA and WorkBeads™40 TREN** media are available in two degrees of substitution, (low-substitution and high substitution,) to allow maximum flexibility when selecting the optimal IMAC conditions. Furthermore, if there is a need for other capacities (substitution levels,) you may use WorkBeads™40 Act media. WorkBeads™40 ACT media allow you to obtain any capacity between specified limits in a simple and reproducible way.

An excellent article describing the use of IMAC media entitled; **"How to use immobilized metal ion affinity chromatography"** is published in Methods: A Companion to Methods in Enzymology 4, 4-13 (1992) by Joy J. Winzerling et.al.

Chelating Groups

There are two different chelators attached to **WorkBeads™40** media. The well known iminodiacetic acid (IDA), Fig 1

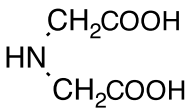

Fig. 1 Iminodiacetic acid (IDA)

and the new chelator - tris(2-ethylaminoethyl)amine (TREN.)

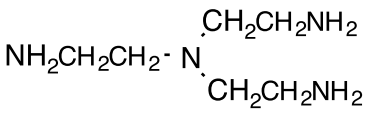

Fig. 2 Tris(2-ethylaminoethyl)amine (TREN)

Metal Ions and Loading

The following metal ions have been used most frequently for IMAC: Zn2+ Cu2+ Ni2+, Fe3+, Co2+, Ca2+ and Al3+ but in principle all metal ions known to interact with proteins can be used. It should be noted that the total capacity for the different gels are specified only for Cu2+ and will vary slightly for other metal ions.

A 50 mM solution of a suitable metal is prepared in distilled water. Some care has to be taken when selecting the loading buffer. The concentration of metal ion will be rather high when absorbed to the gel and precipitation may occur. Normally a 0.1 M sodium acetate buffer pH 5.5 can be used. The metal salt solution is added through a sample loop by repeated injection until the gel is fully loaded.

Adsorption and De-sorption Conditions

These conditions vary of course with the separation problem. Adsorption solvents are normally aqueous but organic solvents in low concentration can also be used. Depending on the nature of the chelator both electrostatic forces and hydrophobic interaction may be involved and care has to be taken with the ionic strength of the buffer. Buffers containing groups with affinity for the metal ion should be avoided (e.g. imidazole.)

Protein desorption is either done by change of the pH or by competition for the metal binding sites between the protein and an another compound like ammonium salts or imidazole buffer.

Removal of Metal Ions

Many metal ions undergo redox reactions and this may cause deviations during storage of the gel. If the gel is not going to be used for a long time we recommend to remove the metal ions. This is easily done with 0.1 M solution of ethylenediamine tetra acetic acid (EDTA) either through repeated injection via the sample loop or directly through the pump.

Applications

Not all proteins behave the same and this is the reason for having four different varieties of IMAC chemistries. Start with IDA high as this will have the best capacity and works well for most of the proteins. If proteins are difficult to desorb or are eluted with less activity it is an indication of too strong binding, in this case try IDA low. If this will not give you the result try TREN High followed by TREN Low. In this way you will have good probability to find the media that will fit your application.

Separation Media Characteristics

	WorkBeads 40 IDA	**WorkBeads 40 TREN**
Agarose Content %	7.4 - 7.8	7.4 - 7.8
Chelating Group	Iminodiacetic acid (IDA)	Tris(2-ethylaminoethyl)amine (TREN)
Metal Ion Capacity *μ*eqv Cu2+ /mL	10-20 IDA^{low} 40-50 IDA^{high}	10-20 $TREN^{low}$ 50-60 $TREN^{high}$
Particle Size, (microns)	35 - 45	35 - 45
Max Flow Rate at 20cm Bed Height and 5 Bar, cm/h	> 500	> 500
pH Stability	pH 2 - 14	pH 2 - 14
Solvent Stability After Coupling the Ligand	100% methanol, 100% ethanol, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitril, 70% formic acid, 30% trifluoroacetic acid	

ORDERING INFORMATION

Product Name	Volume	Article Number
WorkBeads™40 IDA high	Bulk Media – 25 ml	**40 601 001**
WorkBeads™40 IDA high	Bulk Media – 150 ml	**40 601 003**
WorkBeads™40 IDA high	Bulk Media – 1 L	**40 601 010**
WorkBeads™40 IDA low	Bulk Media – 25 ml	**40 602 001**
WorkBeads™40 IDA low	Bulk Media – 150 ml	**40 602 003**
WorkBeads™40 IDA low	Bulk Media – 1 L	**40 602 010**
WorkBeads™40 TREN high	Bulk Media – 25 ml	**40 603 001**
WorkBeads™40 TREN high	Bulk Media – 150 ml	**40 603 003**
WorkBeads™40 TREN high	Bulk Media – 1 L	**40 603 010**
WorkBeads™40 TREN low	Bulk Media – 25 ml	**40 604 001**
WorkBeads™40 TREN low	Bulk Media – 150 ml	**40 604 003**
WorkBeads™40 TREN low	Bulk Media – 1 L	**40 604 010**

All media are preserved in 22% ethanol.

To purchase Bio-Works separation media contact your local distributor. Alternatively, you may email, fax or phone Bio-Works directly at:

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For more information about Bio-Works Company Ltd., please visit our website at: www.bio.works.net.

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