Instructions for use

WorkBeads 40 IMAC media

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**

UNPACKING AND INSPECTION

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier or directly to Bio-Works. Co. Ltd.

STORAGE

WorkBeads 40 IMACmedia are supplied as aqueous suspensions containing 22% ethanol as preservative. The gels can be stored at room temperature. We recommend to add ethanol or sodium azid, if stored in buffer, to prevent bacterial growth.

PACKING OF BULK MEDIA

The ligands are attached to WorkBeads 40 i.e. beads that have a mean bead size of 40 micrometer. The beads are cross-linked with a proprietary method that results in very rigid beads that can take pressure of several bars and run at high flow rates. Follow this general advice when packing a column as well as the column manufacture's specific instructions. Preferably, use a column with an adjustable adaptor. In some instances a packing reservoir or column extension may be used.

Make 50% slurry of the gel and pour into the column. Pack the media with a downward flow higher that the intended operational flow or maximum 10 cm/min linear flow rate. When the bed height is constant, stop the flow and place the adjustable adaptor on top of the packed bed and squeeze it down approximately 2 mm into the bed (axial compression).

Equilibrate the column with a few column volumes of buffer and the column is ready for use.

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METAL IONS AND LOADING

The following metal ions have been used most frequently for IMAC: Zn^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+}, Ca^{2+} and Al^{3+} but in principle all metal ions known to interact with proteins can be used.

A 50 mM solution of a suitable salt 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 adsorbed to the gel and precipitation may occur. Normaly a 0.1 M sodium acetetate 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 DESORPTION CONDITIONS

These conditions varies of course with the separation problem. Adsorbtion 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 to the ionic strength of the buffer. Buffers containing groups with affinity for the metal ion should be avoided.

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 imidazol buffer.

REMOVAL OF THE METAL ION

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 longer time we recommend to remove the metal ion. This is easily done with 0.1 M solution of ethylendiamine tetraacetic acid (EDTA) either through repeated injection via the sample loop or directly through the pump.