WorkBeads 40/1000 ACT
WorkBeads 40/10 000 ACT

WorkBeads™ 40/1000 ACT and WorkBeads 40/10 000 ACT are pre-activated resins that enable easy and reliable coupling of proteins, peptides and low-molecular weight substances for the preparation of customized chromatography resins or enzyme reactors. The bromohydrin active group reacts with thiol, amino and hydroxyl groups. Two different resin porosities are available to facilitate optimized coupling of ligands of different sizes, or to optimize the prepared affinity resin for target molecules of different sizes.

- Easy and reliable coupling procedure
- Stable covalent linkage
- Suitable for coupling of ligands containing thiol, amino and hydroxyl groups

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology, from research to production scale purification, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations that require optimal capacity and purity.
WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT pre-activated resins are a reliable starting material for the preparation of customized chromatography resins. A wide number of organic molecules and biomolecules containing thiol- (i.e., sulfhydryl), amino- or hydroxyl-groups can be coupled covalently by nucleophilic displacement to the agarose matrix activated according to the well-documented bromohydrin method (see Figure 1).

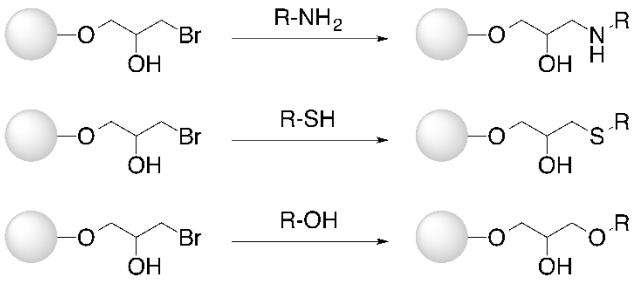

Figure 1. Reaction scheme for coupling a, from top to bottom, primary amine, thiol and alcohol to bromohydrin activated resin.

The main characteristics of the pre-activated WorkBeads resins are shown in Table 1.

For additional information about the derivatization of pre-activated resins, see instruction IN 40 400 010.

Table 1. Main characteristics of WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT resins

	WorkBeads 40/1000 ACT	WorkBeads 40/10 000 ACT
Target substance	Small molecules and peptides	Small molecules, peptides, proteins, e.g., Immunoglobulins
Target groups	Thiol, amino, and hydroxyl groups	
Matrix	Rigid, highly cross-linked agarose	
Average particle size[1] (D_{v50})	45 µm	
Reactive groups	Bromohydrin	
Exclusion limit	1 x 10^6 kDa (globular proteins)	10 x 10^6 kDa (globular proteins)
Max flow rate (20 cm bed height and 5 Bar)	700 cm/h	700 cm/h
Reactive-groups content	250 µmol/ml	150 µmol/ml
Chemical stability (before coupling[2])	Buffers pH<8.5;	
Chemical stability (after coupling[3])	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time	
pH stability[3]	2 – 13 (after coupling)	
Storage[4]	2 to 25 °C in 20 % ethanol	

1. The median particle size of the cumulative volume distribution.
2. Avoid substances containing thiol and amino groups. Substances containing hydroxyl groups will only react if deprotonated. The unreacted resin is generally stable in alcohols at neutral pH.
3. Agarose matrix and linker. Stability of the coupled substance may differ.
4. The choice of storage conditions for the coupled resin depends on the nature of the ligand.

Applications

The design of customized chromatography resins often requires the use of methods for covalent coupling of a ligand to the matrix (non-functionalized resin). Coupling is generally done in three steps; activation, coupling and blocking of unused activated groups. WorkBeads 40/1000 ACT and WorkBeads 40/10000 ACT are pre-activated to have a bromohydrin group that is reactive towards primary amines, thiol- (sulfhydryl), hydroxyl-, or histidyl residues. Most substances containing one or more of these groups can be coupled to the resin. The coupling reaction results in a stable covalent bond. The blocking step is required to eliminate further coupling of substances that are in contact with the resin during subsequent use of the prepared resin. Blocking is often done using ethanolamine or ß-mercaptoethanol.

The bromohydrin coupling method does not introduce additional charges. The coupling is done at room temperature in aqueous solution, and does not release hazardous chemicals during normal use. The work can be performed on the lab bench as long as the substance to be coupled is not hazardous.

Ligands with free amino and sulfhydryl groups will couple easily overnight in basic pH conditions at room temperature, with constant stirring to keep the resin in suspension. Coupling of substances containing hydroxyl groups require high pH (pH >12) due to the low nucleophilicity of this functionality. The hydroxyl groups need to be deprotonated. Coupling of thiol-containing substances can be done under weakly alkaline conditions.

In general, the coupling yield will increase at higher pH. However, hydrolysis of the bromohydrin groups will compete with the coupling reaction at high pH values. An optimium value for pH is often observed for coupling yield. Users should develop a specific procedure optimized for the coupling reaction and for the stability of the substance to be immobilized.

WorkBeads 40/1000 ACT and WorkBeads 40/10000 ACT are suitable for the preparation of affinity chromatography resins or enzyme reactors. The porosity of these two resin differs (see Exclusion limits in Table 1). A higher porosity for the prepared affinity resin may be required if the ligand to be attached or the target is large. A lower porosity can be used if the ligand or the target is small.

Standard coupling protocol

Coupling conditions are listed in Table 2.

1. Wash the resin with deionized water on a glass filter, and dry using suction until drops stop coming.
2. Dissolve the ligand to be coupled in a suitable coupling buffer, or in water.
3. Add the ligand solution to the resin in a final resin slurry concentration of 40-60%.
4. Incubate overnight at room temperature with stirring.
5. Wash with coupling buffer or deionized water to remove unreacted ligand. Suction dry the resin.
6. Block the remaining reactive groups by incubation at room temperature overnight with suitable blocking reagent, for example 1 M ethanolamine-HCl, pH 9.5, with stirring.
7. Wash with coupling buffer or deionized water to remove unreacted blocking reagent.
8. If the resin is not being used for the intended application immediately transfer it into 20% ethanol for storage.

The resin can be packed in a chromatography column or be used as a suspension.

For more detailed coupling instructions, please refer to instructions IN 40 400 010.

Table 22. Type of ligand and most suitable coupling conditions

Type of ligand	Functional group of ligand	Coupling conditions
Organic molecules, peptides	Thiol (Sulfhydryl) (-SH)	pH >7 and higher
Organic molecules, peptides	Amino[1] (-NH_2, -NH, -N)	pH >7 and higher[2]
Proteins, polypeptides	Thiol (Sulfhydryl) (-SH)	pH 7 and higher
Proteins, polypeptides	Primary amino (-NH_2)	Carbonate buffer pH 8 - 8.5[3]
Substance stable at high pH	Hydroxyl (-OH)	pH >12 [4, 5]

1. Substances containing primary, secondary and tertiary amines.
2. Alkaline ligands used in excess may give high enough pH for the reaction to take place. Dissolve it in distilled water and let the basicity of the ligand determine the coupling pH.
3. Sufficient coupling without denaturation of sensitive polypeptides and proteins. Coupling reaction at a lower temperature is also possible.
4. High pH is required due to the low nucleophilicity of the hydroxyl group.
5. At this pH hydrolysis of the bromohydrin will compete with the coupling reaction.

Cleaning-in-place

When the ligand-coupled resin is used for purification or in an enzyme reactor contaminants from the sample (feed), e.g., cell debris, lipids, nucleic acids and protein precipitates, may gradually build up in the resin. The severity of this fouling process depends on the type of sample applied to the column, and the pre-treatment of the sample. This contamination may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution and flow properties of the column.

A specific cleaning protocol should be designed for each process according to the type of sample purified and the stability of the ligand attached to the resin. For stable resins, cleaning can often be done overnight with 1 M NaOH, whereas resins with sensitive ligands can often be cleaned using non-ionic detergent.

Storage

Store at 2 to 25°C in 20% ethanol.

The choice of storage conditions and the stability of the coupled resin depend on the nature of the coupled ligand.

Related products

Related product	Pack size[1]	Article number
BabyBio ACT 1 ml[2]	1	45 400 001
	2	45 400 002
	5	45 400 003
	10	45 400 004
BabyBio ACT 5 ml[2]	1	45 400 005
	2	45 400 006
	5	45 400 007
	10	45 400 008
WorkBeads 40/1000 SEC[3]	25 ml	40 300 001
WorkBeads 40/10 000 SEC[3]	25 ml	40 350 001

1. Other pack sizes can be found in the complete product list on our website www.bio-works.com.
2. BabyBio are columns pre-packed with different WorkBeads resins. BabyBio ACT columns are pre-packed with WorkBeads 40/1000 ACT.
3. The resin can be treated with suitable reagent for activation, and used for ligand coupling.

Ordering information

Product name	Pack size	Article number
WorkBeads 40/1000 ACT	50 ml	40 400 001
	300 ml	40 400 003
	1 L	40 400 010
	5 L	40 400 050
WorkBeads 40/10 000 ACT	50 ml	40 450 001
	300 ml	40 450 003
	1 L	40 450 010
	5 L	40 450 050

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products please visit www.bio-works.com or contact us at info@bio-works.com.

Notes:

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden