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 simple 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, and to optimize the prepared affinity resin for target molecules of different sizes.

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

Short protocol

This short protocol is for coupling ligand onto WorkBeads ACT resins. Detailed instructions and recommendations are provided later in this instruction. Recommended coupling buffers are listed in Table 1.

1. Wash the resin with deionized water on a glass filter, suction dry the resin.
2. Dissolve the substance to be coupled (the ligand) in suitable coupling buffer.
3. Add the ligand-solution to the resin.
4. Incubate overnight with agitation.
5. Wash with buffer or deionized water to remove unreacted ligand. Suction dry the resin.
6. Block the remaining reactive groups by incubation overnight under agitation with a suitable blocking reagent, for example 1 M ethanolamine-HCl, pH 9.5.
7. Wash with buffer or deionized water to remove excess blocking reagent.
8. Use the resin for the intended application, or transfer to 20% ethanol for storage.

The resin may be packed in a chromatography column or be used as a suspension in a batch process.

Principle

The development of customized chromatography resins requires methods for covalent attachment of a functional ligand to the matrix. The ligand can be a protein, peptide, carbohydrate, or an organic substance. The WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT resins contain bromohydrin groups that are reactive towards the nucleophilic N, S, or O atoms in primary amines (sulfhydryl, hydroxyl, aldehyde, carboxylic or histidyl groups), in the ligands to be coupled. The nucleophilic displacement reaction occurs at ambient temperature in aqueous solution under mildly alkaline or alkaline conditions to create a stable covalent bond between the resin and the ligand (Figure 1). There is no need for any additional reagent and the coupling does not create any additional charged groups. After ligand coupling, remaining active groups must be blocked. This is carried out by adding a blocking agent which reacts with the remaining bromohydrin groups. Ethanolamine or mercaptoethanol are often used as blocking agent, since the reaction introduce a $–CH_2CH_2OH$ group. For alkaline-stable ligand/resins constructs NaOH can be used instead of a blocking agent to hydrolyse the remaining bromohydrin groups.

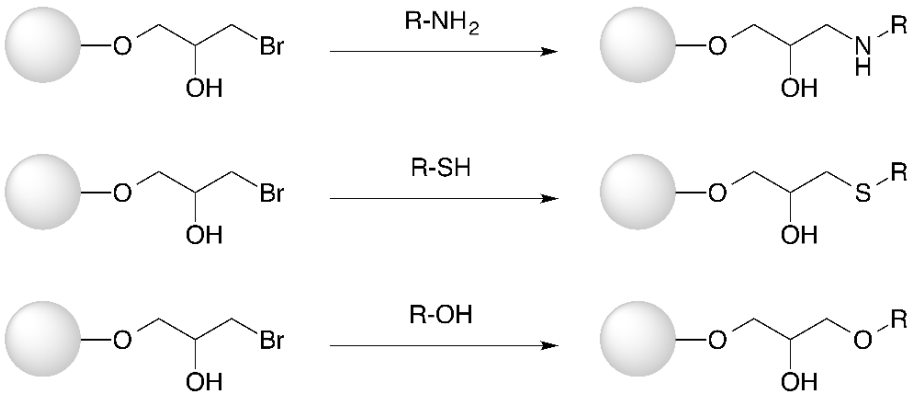

Figure 1. Reaction scheme for coupling a, from top to bottom, primary amine, thiol and alcohol to bromohydrin activated resin.

Instructions

This is a general protocol for ligand coupling of WorkBeads 40/1000 ACT or WorkBeads 40/10 000 ACT. Successful coupling may require further optimization of the conditions for coupling, blocking and washing conditions.

1. Prepare the resin

Wash the required volume of resin 3 times with 3 volumes (resin bed volumes) of deionized water on a glass filter with vacuum suction. Between washes, re-suspend the resin by careful stirring using a soft spatula. After the third wash, maintain the vacuum until the resin cake cracks (suction dry). Weigh the resin and transfer it to a suitable reaction vessel. The reactions can be carried out in a test tube, flask or in a reaction vessel with overhead stirring.

2. Prepare coupling solution with the ligand

Prepare 1 to 2 ml coupling solution per gram of suction dried resin. This corresponds to a slurry concentration in the final reaction mixture of approx. 33 to 50%. Dissolve the required amount of ligand in 100 mM sodium carbonate buffer, pH 8.5 or other suitable solution (See Table 1 and *Optimization*).

General recommendations: For protein and peptide, coupling it is typical to use 0.1 to 20 mg polypeptide per ml suction dried resin. For organic substances with low molecular weight, use 0.1 to 5 equivalents of ligand per single equivalent bromohydrin groups on the resin.

3. Prepare the coupling mixture

Add the entire volume of the prepared coupling solution (containing the dissolved ligand) to the reaction vessel, containing the prewashed, suction dried resin, and start agitation. Avoid magnetic stirrer since the magnet may grind the resin particles against the bottom of reaction vessel destroying the beads and cause fines.

4. Incubate

Leave the reaction to take place overnight at room temperature (approximately 16 hours). A different time may be required (see *Optimization*).

5. Remove free ligand by washing

Transfer the ligand-coupled resin to a glass filter and wash using 3 - 10 volumes of coupling solution (not containing ligand), deionized water or other suitable solution (for buffers, etc., see *Optimization*). Suction dry and transfer the washed ligand-coupled resin back to the reaction vessel.

6. Block the remaining active groups

Add 1 ml blocking reagent solution, for example 1 M ethanolamine-HCl, pH 9.5, per 1 g suction dried ligand-coupled resin. Allow the blocking reaction to take place at room temperature overnight (16 hours) with agitation. Avoid magnetic stirring.

7. Wash to remove the blocking solution

Transfer the blocked resin to a glass filter. Wash with coupling buffer or deionized water until all excess of blocking agent is removed.

8. Transfer to storage solution

Wash the blocked resin with 3 volumes storage solution (e.g., 20% ethanol) or a suitable buffer (see *Maintenance* for suggestions regarding storage).

The ligand-coupled resin is now ready for use.

Table 1. Suggested coupling buffers. Other buffers may be used.

Type of ligand	Functional group of ligand	Coupling conditions
Proteins and peptides	Primary amino (-NH_2)	100 mM sodium carbonate buffer, pH 8 - 8.5[1]
	Sulphydryl (-SH)	200 mM sodium phosphate, pH 8 Higher pH (within the protein stability range)
Organic molecules	Amino (-NH_2, -NH, -N)	Coupling pH determined by the ligand basicity[2]
	Sulphydryl (-SH)	pH 7 and higher
Carbohydrates	Hydroxyl (-OH)	pH > 12 [3, 4]

1. Sufficient coupling without denaturation of sensitive polypeptides and proteins. Coupling reaction at lower temperature is also possible.
2. When the ligand is used in excess, dissolve it in deionized water and let the basicity of the ligand determine the coupling pH.
3. High pH is required due to the low nucleophilicity of the hydroxyl group.
4. Note: At this pH, cross-linking and hydrolysis will compete with the coupling reaction.

Optimization

Users should develop a specific coupling procedure suitable for the nature and stability of the specific ligand and the requirements of the intended application. There are several factors to take into account in order to optimise the coupling protocol.

Selection of resin

The porosity of the resin used for ligand coupling may affect the amount of ligand that can be immobilized. A high porosity can accommodate larger ligands. On the other hand, high porosity resins have lower available pore wall surface area, which may limit the amount of ligand that can be attached to the resin. The optimum porosity for coupling a high amount of ligand may therefore differ between ligands of different sizes. It should also be noted that the porosity of the final resin (resin with attached ligand) may also affect the binding capacity of the target substance when the ligand-resin is used for affinity chromatography. WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT has a cut-off of approximately 1×10^6 and 1×10^7 Da, respectively. WorkBeads 40/1000 ACT is recommended to be used for the immobilization of most proteins, peptides and low-M_r substances, and WorkBeads 40/10 000 ACT for larger proteins and protein complexes.

Optimization of coupling conditions

Coupling solution

Coupling should be carried out in aqueous, mild alkaline buffered solutions (e.g., using carbonate, borate or phosphate buffers) or in strongly alkaline solutions (e.g., high concentration of NaOH). The buffer substance should not contain any nucleophilic functional groups (e.g., Tris, glycine or Good's buffers) since these compounds will react with the resin and compete with the coupling of the desired ligand. Suggested coupling buffers are provided in Table 1. Due to possible pH reduction, as a result of the release of HBr during the reaction, it is generally recommended to use a high buffer concentration or a high enough concentration of NaOH to neutralize the released HBr.

Always check, and if needed, adjust the pH after dissolving the ligand since it may change the pH upon dissolution. Sodium hydroxide and hydrochloric acid may be used to adjust the pH of solutions, but precautions should be taken into account in order to avoid denaturation when working with protein ligands.

The ligand to be coupled should be fully soluble in the coupling solution. The addition of organic solvents may be needed to dissolve the ligand. Dimethylformamide and dioxane may be used to up to 50% of the final mixture. If the ligand is a protein make certain that it is stable in the coupling solution.

pH

The coupling reaction can be carried out in the pH range 7 to 14. Ligands carrying amine or sulfhydryl (thiol) groups can often be coupled in the pH range 7 to 10, whereas coupling via hydroxyl groups requires higher pH (pH > 12) to deprotonate the hydroxyl group. Although the coupling yield will increase at higher pH, the cross-linking and hydrolysis will compete with the coupling reaction at pH higher than 12. The chemical stability and the solubility of the ligand limits the maximum pH that can be used.

Temperature

Coupling can be carried out from 4 to 40 °C. The coupling decreasing at higher temperatures. Direct heating should be avoided. The stability of the ligand limits the maximum temperature that can be used. For protein coupling it is recommended to use room temperature. Lower temperatures may be required, but will reduce mass transfer and reaction rate, thus require longer reaction times.

Time

The time for the reaction depends on the properties of the ligand, the pH and the temperature of the coupling reaction. A reaction time of 16 hours at ambient temperature (20 to 25°C) is a general recommendation. Optimum reaction times may vary from 2 to 48 hours. The determination of a suitable reaction time can be made by following the progress of the reaction by analyzing the change in ligand concentration of the free solution:

1. Take out a small volume of the reaction mixture (resin suspension).
2. Centrifuge at 100 x *g* for 2 minutes.
3. Analyze the presence of the ligand in the supernatant. The reaction is complete when there is no ligand left in the supernatant, or when the ligand concentration has stabilized. (When no more ligand can be coupled).

Removal of free ligand after coupling

After coupling and blocking, it is important to remove any remaining ligand and blocking agent that has not been coupled to the resin. This will avoid subsequent leakage of these substances from the ligand-coupled resin. Multiple washes with alternating high and low pH, and high and low ionic strengths are recommended, especially when the ligand is a biomolecule such as a protein or a peptide. Organic solvent washes should be considered if the ligand is a low molecular weight organic substance with limited solubility in aqueous solutions.

Maintenance

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.

Storage

WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT resins are supplied as aqueous suspensions containing 20% ethanol as preservative. The activated form of the resins are stable at pH 4 to 7 at 2 to 25°C.

After ligand coupling the stability of the ligand-coupled resin will usually be dependent on the chemical stability of the ligand. The ligand is often more stable when coupled rater than when in solution. Although it is often possible to store the ligand coupled-resin in 20% ethanol, alternative storage solutions may need to be selected to optimize stability. If 20% ethanol cannot be used then addition of antimicrobial agents may be useful. If the coupled ligand is a peptide or protein, addition of proteolytic enzyme inhibitors should be considered. Sensitive ligand-coupled resins should be stored at 2 to 4 °C.

Additional information

Product information

	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	Thiol, amino, and hydroxyl groups
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm	45 µm
Reactive groups	Bromohydrin	Bromohydrin
Exclusion limit	1 x 10^6 Da (globular proteins)	1 x 10^7 Da (globular proteins)
Max flow rate[2]	600 cm/h	600 cm/h
Reactive-groups content	200 µmol/ml	200 µmol/ml
Chemical stability (before coupling[3])	Buffers pH < 8.5	Buffers pH < 8.5
Chemical stability (after coupling[4])	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[4]	2 - 13 (after coupling)	2 - 13 (after coupling)
Storage[5]	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Determined in water using a 10 x 300 mm column
3. Avoid substances containing thiol and amino groups. Substances containing hydroxyl groups will only react if deprotonated. The unreacted resin is stable in alcohols at neutral pH.
4. Agarose matrix and linker. Stability of the coupled substance may differ.
5. The choice of storage conditions for the coupled resin depends on the nature of the ligand.

Intended use

WorkBeads ACT resins are intended for research, process development and industrial scale purifications. WorkBeads ACT resins shall not be used for preparation of material for clinical or diagnostic purpose.

Safety

Please read the associated Safety Data Sheets (SDS) for WorkBeads ACT resins and any reagent to be used, as well as the safety instructions for any equipment to be used.

Related products

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

1. Other pack sizes can be found in the complete product list on www.bio-works.com
2. BabyBio ACT columns are prepacked with WorkBeads 40/1000 ACT.

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

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