

## BabyBio ACT

The ready-to-use BabyBio™ ACT columns are prepacked with WorkBeads™ 40/1000 ACT resin and are available in two column sizes: 1 ml and 5 ml. WorkBeads 40/1000 ACT pre-activated resin enable easy and reliable coupling of proteins, peptides and low-molecular weight substances for the preparation of customized chromatography resins. The bromohydrin active group reacts with thiol, amino and hydroxyl groups.

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



### Resin description

The BabyBio ACT columns are prepacked with WorkBeads 40/1000 ACT pre-activated resin. This resin is a reliable starting material for the preparation of customized chromatography resins. A wide number of organic molecules and biomolecules containing sulfhydryl-, amino- or hydroxyl-groups can be coupled covalently by nucleophilic displacement to the agarose matrix according to the well-known bromohydrin activation method (Figure 1).

The main characteristics of BabyBio ACT columns are shown in Table 1. For more detailed instructions of how to use BabyBio ACT, see instruction IN 45 400 010.

### Column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. The ready-to-use BabyBio columns are delivered with an inlet plug, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10–32) for 1/16" o.d. tubing (standard HPLC PEEK tubing).

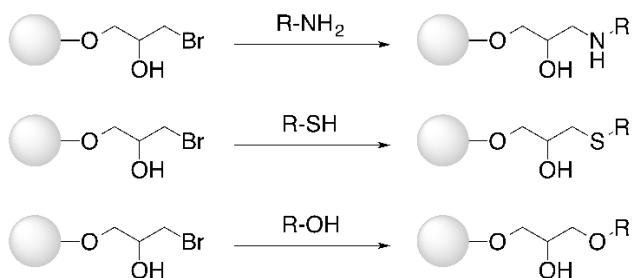


Figure 1. Reaction scheme for coupling from top to bottom, primary amine, thiol and alcohol to bromohydrin activated resin.

Table 1. Main characteristics of the BabyBio ACT 1ml and BabyBio ACT 5 ml columns.

	BabyBio ACT
Resin	WorkBeads 40/1000 ACT
Matrix	Rigid, highly cross-linked agarose
Average particle size <sup>1</sup> (D <sub>V50</sub> )	45 µm
Reactive group	Bromohydrin
Reactive-groups content	200 µmol/ml resin
Column volume	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate	
BabyBio 1 ml	1 ml/min (150 cm/h)
BabyBio 5 ml	5 ml/min (225 cm/h)
Maximum flow rate <sup>2</sup>	
BabyBio 1 ml	5 ml/min (780 cm/h)
BabyBio 5 ml	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability (before coupling <sup>3</sup> )	Buffers pH < 8.0
Chemical stability (after coupling <sup>4</sup> )	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 <sup>4</sup>	2 - 13 (after coupling)
Storage <sup>5</sup>	2 to 25°C in 20% ethanol (before coupling)

1. The median particle size of the cumulative volume distribution.

2. Aqueous buffers at 20°C. Decrease the maximum flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the maximum flow rate at 4°C), or by additives (e.g. use half of the maximum flow rate for 20% ethanol).

3. 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.

4. Agarose matrix and linker. Stability of the coupled substance may vary.

5. The choice of storage conditions for the coupled resin depends on the nature of the ligand. Often 20% ethanol can be used as bacteriostat.

## Applications

The design of customized chromatography resins often requires various covalent coupling methods can be used for the coupling of a ligand to the matrix (non-functionalized resin). Coupling is generally carried out in three steps; activation, coupling and blocking of unused activated groups.

WorkBeads 40/1000 ACT is 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 carried out using ethanolamine or β-mercaptoethanol by reaction of the amine or thiol group.

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 carried out 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. Coupling of substances containing hydroxyl groups require high pH (pH > 12) due to the low nucleophilicity of this functionality and the hydroxyl groups need to be deprotonated. Coupling of thiol-containing substances can be carried out under weakly alkaline conditions.

In general, coupling yield will increase at higher pH. However, hydrolysis of the bromohydrin groups will compete with the coupling reaction at high pH values. A pH optimum 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.

After the coupling, it is recommended to deactivate any remaining reactive groups using mercaptoethanol or ethanolamine.

### Standard coupling protocol

Coupling conditions are listed in Table 2.

1. Dissolve the ligand to be coupled in a suitable coupling buffer, or in water.
2. Wash the column with suitable coupling buffer, or in water.
3. Add the ligand solution to the column. If the ligand solution has the same volume as the column this is easily done using a syringe. If the ligand solution volume is larger than the column volume the solution can be re-circulated using a pump.
4. Allow time for the reaction to take place. The reaction time is ligand dependent and needs to be optimized. A general recommendation is to leave the reaction to take place overnight at room temperature (approximately 16 hours).
5. Wash the column with coupling buffer or deionized water to remove unreacted ligand.
6. Block the remaining reactive groups by incubation at room temperature overnight with suitable blocking reagent. For example, by using 1 M ethanolamine-HCl, pH 9.5.
7. Wash the column with coupling buffer or deionized water to remove unreacted blocking reagent.
8. If the column is not quickly used for the intended application immediately equilibrate with 5 CV of storage solution (e.g., 20% ethanol) or a suitable buffer. Close the column using the cap and plug.

For more detailed coupling instructions, please refer to instructions IN 45 400 010.

Table 2. 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 <sup>1</sup> (-NH <sub>2</sub> , -NH, -N)	pH > 7 and higher <sup>2</sup>
Proteins, polypeptides	Thiol (Sulfhydryl) (-SH)	pH 7 and higher
Proteins, polypeptides	Primary amino (-NH <sub>2</sub> )	Carbonate buffer pH 8 - 8.5 <sup>3</sup>
Substance stable at high pH	Hydroxyl (-OH)	pH > 12 <sup>4, 5</sup>

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.

### Scale-up

Scale-up can be carried out conveniently from a 1 ml column to a 5 ml column. If increased capacity is required several columns can be coupled in series (column stacking). Note that the backpressure will increase proportionally to the resin bed height (use up to a maximum of 5 columns).

Further scale-up can be achieved by coupling and packing bulk WorkBeads 40/1000 ACT resin in larger columns (see *Related products*).

### Cleaning-in-place

When the ligand-coupled resin is used for purification impurities 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 depends on the type of sample applied to the column, and the pre-treatment of the sample. These adsorbed impurities 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 coupled resins, cleaning can often be done overnight with 1 M NaOH, whereas resins with sensitive ligands can often be cleaned using non-ionic detergent.

## Equipment

Prepacked BabyBio ACT ready-to-use columns can be used with most standard liquid chromatography equipment. Purification can also be carried out using a syringe connected to the column by a luer or a std HPLC connector.

## Storage

Equilibrate the column in 20% ethanol and close it securely using the plug and cap included. Store at 2 to 25°C.

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

## Related products

Product name	Pack size <sup>1</sup>	Article Number
<b>Bulk resins</b>		
WorkBeads 40/1000 ACT	50 ml	40 400 001
WorkBeads 40/1000 ACT	300 ml	40 400 003
<b>Accessories</b>		
Column Plug Male 1/16"	10	70 100 010
Column Cap Female 1/16"	10	70 100 020

1. Other pack sizes can be found in the complete product list on our website [www.bio-works.com](http://www.bio-works.com)

## Ordering information

Product name	Pack size	Article number
BabyBio ACT 1 ml	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	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

Orders: [sales@bio-works.com](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products please visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)



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