Bio-Works

INSTRUCTION

GoBio Mini ACT

The ready-to-use GoBio™ Mini 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

Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced according to ISO 9001:2015, and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

The GoBio prepacked column family has been developed for convenient, reproducible, and rapid results and can be used for small scale purification and all the way up to process development and full-scale manufacturing.

Safety

Please read the Safety Data Sheet (SDS) for WorkBeads 40/1000 ACT, and the safety instructions for any equipment to be used.

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to complaints@bio-works.com

Short protocol

This short protocol describes the coupling ligand to WorkBeads resin in a GoBio Mini ACT column. Detailed instructions and recommendations are given later in this document. Recommended coupling buffers are listed in Table 2.

1. Wash the column with deionized water.
2. Dissolve the substance to be coupled (the ligand) in suitable coupling buffer.
3. Apply the ligand solution on the column.
4. Incubate overnight. Alternatively, recirculate ligand solution through the column using e.g., peristaltic pump.
5. Wash with buffer or deionized water to remove unreacted ligand.
6. Block the remaining reactive groups by incubation overnight with suitable blocking reagent, for example 1 M ethanolamine-HCl, pH 9.5. Equilibrate the column with 10 column volumes of blocking solution.
7. Wash with buffer or deionized water to remove excess blocking reagent.
8. Use the column for the intended application or equilibrate the column with 5 CV of 20% ethanol for storage. Close the column using the included cap and plug.

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 resin 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 ß-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.

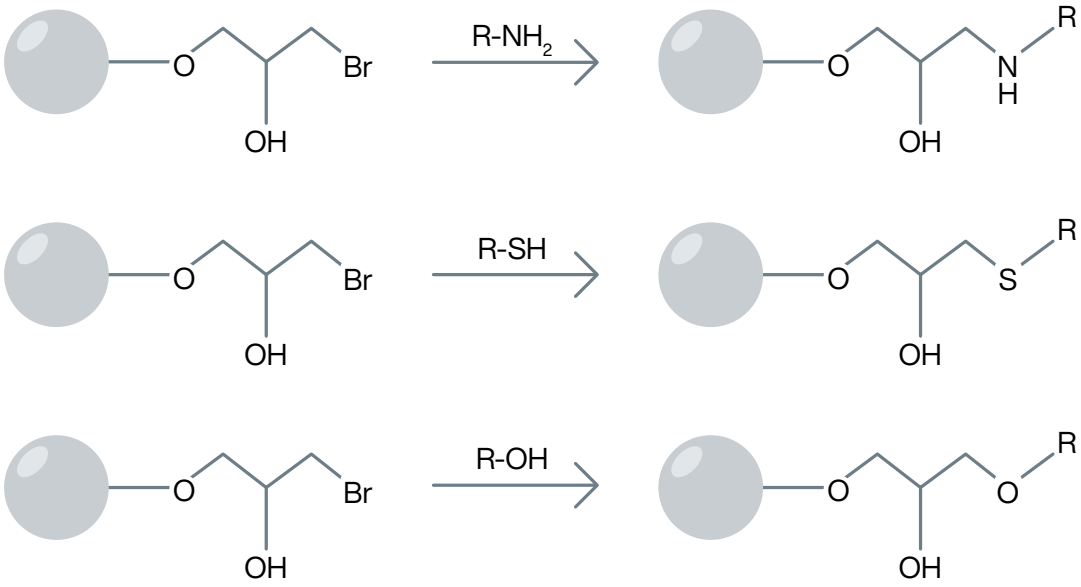

Figure 1. Reaction scheme for coupling a, from top to bottom, primary amine, thiol and alcohol to bromohydrin activated resin.

Instructions

This is a general protocol for ligand coupling on GoBio Mini ACT column. Successful coupling may require further optimization of the conditions for coupling, blocking and washing.

Note: To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

1. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 2. **Note:** It is of high importance to cut off the tip at the very end of the cone, preferable using a scalpel. Incorrect removal of the end piece will affect the performance of the column.

Connect the column to your equipment using the recommended connectors shown in Table 1. Fill the equipment with deionized water or buffer and make drop-to-drop connection with the column to avoid getting air into the column.

Figure 2. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 1. Recommended connectors for coupling GoBio Mini columns to the equipment of choice.

Equipment	Accessories for connection
Syringe	Female luer/male coned 10 – 32 threads
Chromatography system	Fingertight connectors (coned 10 – 32 threads) for 1/16" o.d. tubing

2. Remove the storage solution

The column contains 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV deionized water or coupling buffer. Avoid flow rates higher than 2 mL/min for GoBio Mini 1 mL columns or 10 mL/min for GoBio Mini 5 mL columns before the storage solution has been removed to avoid overpressure due to the relatively high viscosity of the 20% ethanol solution.

3. Equilibrate the column

Equilibrate the column using 5 CV (column volumes) of coupling solution (without ligands).

4. Prepare the ligand solution

Dissolve the required amount of ligand in 100 mM sodium carbonate buffer, pH 8.5 or other suitable solution, see Table 2 and see "Optimization". For stable ligands higher pH values can be used. At high pH values the hydrolysis of the bromohydrin groups will compete with coupling of the ligand.

General recommendations: For coupling of proteins and peptides it is typical to use 0.1 mg to 20 mg polypeptide per mL resin. For expensive organic substances with low molecular weight, use 0.1 to 5 equivalents of ligand per single equivalent bromohydrin groups on the resin. For cheap ligands an excess of 10 equivalents or more can be used. If the ligand solution is going to be loaded on the column using a syringe the coupling solution should not be over 1.2 mL (1 mL column) and 5.4 mL (5 mL column). If the sample easily precipitates and it is preferred to use a larger coupling solution a pump and recirculation can be used.

5. Load the ligand solution to the column and incubate

Using a syringe: Attach a syringe filled with the coupling solution to the top of the column using a female luer/male coned 10 – 32 threads. Make the connection drop-to-drop not introducing any air into the column. When all ligand solution has been loaded onto the column leave the syringe in place and close the column with the included plug. Incubate overnight at room temperature (approximately 16 hours). A different incubation time may be required, see "Optimization".

Using a pump: Fill the pump and tubings with ligand solution and connect drop-to-drop to the column. Recirculate the solution with low flow during the incubation overnight at room temperature (approximately 16 hours). A different incubation time may be required, see "Optimization".

5. Wash out uncoupled ligand

Wash the column with 5 CV coupling solution (not containing ligand), deionized water or other suitable solution (for buffers, etc., see "Optimization".

6. Deactivation of remaining active groups

Deactivate any excess active groups that have not coupled to the ligand by washing the column with 5 CV blocking reagent solution, for example 1 M ethanolamine-HCl, pH 9.5 and leave the column filled with this solution for 4 hour or overnight. A different blocking time may be required, see "Optimization".

7. Wash out the blocking reagent solution

Wash the column with 5 CV coupling buffer or deionized water to remove the blocking agent solution. The ligand coupled column is now ready for use.

8. Column storage

If the column is not being used directly, equilibrate with 5 CV storage solution (e.g., 20% ethanol) or a suitable buffer, see "Maintenance" for suggestions regarding storage. Close the column using the cap and plug (included).

Table 2. Suggested coupling buffers. Other buffers can possible be used.

Type of ligand	Functional group of ligands	Coupling conditions
Proteins and peptides	Primary amino (-NH_2) Sulphydryl (-SH)	100 mM sodium carbonate buffer, pH 8 – 8.5[1] 200 mM sodium phosphate, pH 8 Higher pH (within the protein stability range)
Organic molecules	Amino (-NH_2, -NH, -N) Sulphydryl (-SH)	Coupling pH determined by the ligand basicity[2] pH 6.5 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.

Scale-up

GoBio Mini columns are easily connected without accessories. Up to five columns may be connected in series (column stacking). The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be subjected to a higher internal pressure from the added pressure drops from downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid exceeding the maximum pressure limit onto the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 3. Remember always to take the system fluidics contribution to the pressure into account.

Table 3. Recommended maximum pressure settings for GoBio Mini columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

No. of columns in series	Max pressure GoBio Mini 1 mL (bar)	Max pressure GoBio Mini 5 mL (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10[1]
5	15	10[1]

[1] The maximum pressure is defined by the column hardware maximum pressure

For columns larger than 20 mL, it is recommended to pack a single column using bulk resin or using a prepacked column from the GoBio prepacked column family, as the limitations of column stacking will then impact chromatographic performance.

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Optimization

In most cases the general recommendation will be efficient for preparing customized resins, but in some cases a specific coupling procedure suitable for the nature and stability of the specific ligand and the requirement of the intended application is needed. There are several factors to have in mind when optimising the coupling protocol.

Optimization of coupling conditions

Coupling solution

Coupling should be carried out in aqueous, mild alkaline buffered solutions (e.g., 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 2. Due to possible pH reduction and 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 to avoid denaturation when working with protein ligands.

The ligand to be coupled should be fully soluble in the coupling solution. 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 sure 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 which can be used.

Temperature

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

Time

The time for the reaction depends on the properties of the ligand and the pH and temperature of the coupling reaction. A reaction time of 16 hours at ambient temperature (20 to 25°C) is a general recommendation. Reaction times from 2 to 48 hours may be useful.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification with for example ion exchange chromatography. This can be carried out quickly and easily in lab-scale using GoBio Mini Dsalt 1 mL, GoBio Mini Dsalt 5 mL, GoBio Prep 16x100 Dsalt (20 mL) and GoBio Prep 26x100 Dsalt (53 mL) columns depending on sample volumes, see “Related products”. These columns are also very useful alternatives to dialysis or when samples need to be processed rapidly to avoid degradation. For larger sample volumes, prepacked GoBio Prod columns starting from 1 L are available or diafiltration can be used.

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Maintenance of the column

Cleaning

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 process 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 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

The unused GoBio Mini ACT columns are stable in 20% ethanol at 2 to 25°C. The unreacted resin is generally stable in alcohols at neutral pH (buffers pH < 8.0). Close it securely using the included plug and cap.

After ligand coupling, the stability of the ligand-coupled resin will usually be dependent on the stability of the ligand. The ligand is often more stable after coupling than in solution. Although it is often possible to store the ligand-coupled resin in 20% ethanol, alternative storage solutions may have to be selected to optimize stability. If 20% ethanol cannot be used, addition of antimicrobial agents may be useful. Sensitive ligand-coupled resins should be stored at 2 to 4 °C.

Product descriptions

	GoBio Mini ACT
Resin	WorkBeads 40/1000 ACT
Matrix	Rigid, highly cross-linked agarose
Average particle size1 (Dv50)	45 µm
Reactive group	Bromohydrin
Reactive-groups content	200 µmol/mL resin
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[2] GoBio Mini 1 mL GoBio Mini 5 mL	 0.25 – 1 mL/min (37 – 150 cm/h) 1.25 – 5 mL/min (56 – 225 cm/h)
Maximum flow rate[3] GoBio Mini 1 mL GoBio Mini 5 mL	 5 mL/min (780 cm/h) 20 mL/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability (before coupling[4])	Buffers pH < 8.0
Chemical stability (after coupling[5])	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[5]	2 – 13 (after coupling)
Storage[6]	2 to 25°C in 20% ethanol (before coupling)

1 The median particle size of the cumulative volume distribution.
2 Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
3 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).
4 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.
5 Agarose matrix and linker. Stability of the coupled substance may vary.
6 The choice of storage conditions for the coupled resin depends on the nature of the ligand. Often 20% ethanol can be used as bacteriostat.

GoBio prepacked column family

GoBio prepacked column family is developed for convenient, reproducible and fast results and includes columns with different sizes and formats.

GoBio Mini 1 mL and GoBio Mini 5 mL for small scale purification and screening using a shorter packed bed.

GoBio Screen 7x100 (3.8 mL) for reproducible process development including fast and easy optimization of methods and parameters.

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

GoBio Prod 80x200 (1 L), GoBio Prod 130x200 (2.7 L), GoBio Prod 200x200 (6 L), GoBio Prod 240x200.

(9 L) and GoBio Prod 330x250 (21.4 L) for production-scale purifications.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 40/1000 ACT	50 mL 300 mL	40 400 001 40 400 003
WorkBeds 40 /10 000 ACT	50 mL 300 mL	40 450 001 40 450 003
WorkBeads Dsalt	300 mL	40 360 003
Accessories		
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 www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Mini ACT 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 400 001 45 400 003 45 400 004
GoBio Mini 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 400 005 45 400 007 45 400 008

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

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

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