EconoFit Macro-Prep t-Butyl and EconoFit Macro-Prep Methyl Hydrophobic Interaction Chromatography Columns, 1 ml

Instruction Manual

Catalog #12009277
12009278

Please read the instructions in this manual prior to using EconoFit Macro-Prep t-Butyl and EconoFit Macro-Prep Methyl Hydrophobic Interaction Chromatography Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit Macro-Prep t-Butyl and EconoFit Macro-Prep Methyl Hydrophobic Interaction Chromatography (HIC) Columns are convenient, disposable, prepacked low-pressure chromatography columns. They facilitate both increased run-to-run uniformity and high purity of proteins through the column design and novel resin technology. Compatible with most aqueous buffers commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

These columns are designed specifically for intermediate purification steps that remove host-cell contaminants from partially purified targets. Due to their rigidity and unique surface chemistry, these resins are particularly suited for HIC operations requiring high throughput and high recovery of target. Both Macro-Prep t-Butyl and Methyl HIC Resins operate on a mechanism of interaction that is based on hydrophobicity and charge. The t-butyl and methyl groups are mildly hydrophobic. Depending on the chosen pH of loading and elution buffers, the carboxyl groups can be exploited to ionically repel target molecules while the hydrophobic groups retain contaminants. This is an ideal strategy to minimize product losses commonly experienced with HIC media due to denaturation of proteins when exposed to hydrophobic surfaces.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns, which are supplied ready for use in convenient 1 and 5 ml sizes. They can be quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion), ion exchange, affinity, mixed-mode, and hydrophobic interaction chromotography. See Table 1 for column information and technical specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of products in the EconoFit Column portfolio.

Table 1. EconoFit Macro-Prep t-Butyl and EconoFit Macro-Prep Methyl HIC Column information and specifications.

Property	Description
Functional groups	Macro-Prep t-Butyl: COO^- and t-butyl Macro-Prep Methyl: COO^- and methyl
Ionic capacity	Approximately 120 µeq/ml
Shipping counterion	Cl^-
Hydrophobicity	Mild
Median particle size	50 µm
Size	1 ml bed volume
Bed dimensions	1 ml: 25 mm length x 7 mm inner diameter
Maximum pressure tolerance	72 psi
Recommended flow rate	1 ml: 1–2 ml/min (240–480 cm/hr)
pH stability	1–10
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	20% ethanol
Storage conditions	20% ethanol or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5
Storage temperature	4–40°C
Sanitization	6 M guanidine HCl or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5
Autoclavability	Not autoclavable
Shelf life	1 year at 4°C

Common buffers for hydrophobic interaction chromatography are listed in Table 2.

Macro-Prep t-Butyl and Methyl HIC Resins are also available in larger sizes in bottles. Refer to the ordering information section for more information.

Table 2. Buffers compatible with Macro-Prep t-Butyl and Methyl HIC Resins.

Buffer	Buffering Range, pH
Citric acid	2.6–3.6
Lactic acid	3.6–4.3
Formic acid	3.8–4.3
MES	5.5–6.7
PIPES	6.1–7.5
MOPS	6.5–7.9
Phosphate	6.7–7.6
Bicine	8.2–8.7
HEPES	6.8–8.2
TES	6.8–8.2
Tricine	7.4–8.8

Macro-Prep t-Butyl and Methyl HIC Resins are based on rigid, epoxy-activated methacrylate beads derivatized with t-butyl or methyl groups, respectively, and carboxyl groups (Figure 1).

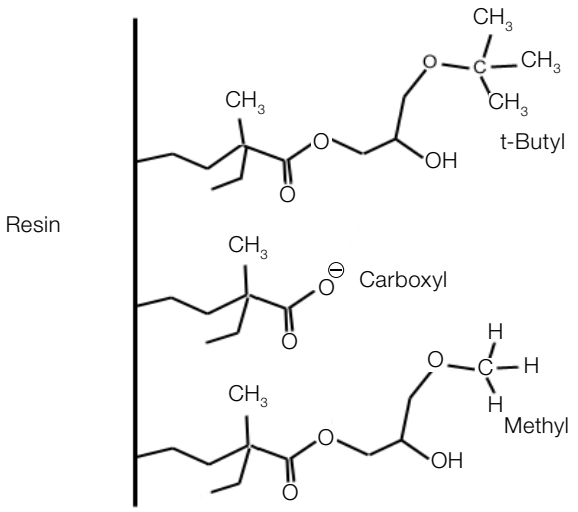

Fig. 1. Macro-Prep HIC Resins. Macro-Prep t-Butyl HIC Resin contains t-butyl and carboxyl groups. Macro-Prep Methyl HIC Resin contains methyl and carboxyl groups.

Section 3
Sample Preparation

For best results, and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter. Adjust salt concentration and pH as necessary for desired binding of target or contaminants. This is best done by mixing the correct amount of liquid concentrate of salt into the sample load and then adjusting the pH.

Section 4
Preparing a Column and Subsequent Purification

Higher salt concentrations are used to enhance hydrophobic interactions. Normally, a column is equilibrated in a high-salt buffer, and the sample is loaded onto the column at the same pH and salt concentration. At small scale, the following salts are commonly used: ammonium sulfate (<pH 8) and 1 M solutions of sodium sulfate, sodium chloride, potassium chloride, or sodium citrate. At larger scale, sodium citrate is a very practical salt. This salt can function as both a buffer and a salt during HIC. Lower molar quantities are required to reach specific ionic concentrations and the somewhat hydrophobic nature of the citrate ion often minimizes product loss due to overly strong product interaction with the media. If the target molecule is in the flowthrough, sodium citrate is often used at concentrations of 500–600 mM for loading.

Sample Load and Adsorption

The sample load is determined empirically by loading and evaluating breakthrough of target or contaminants. Since HIC is an adsorption technique, the sample volume is not a critical factor. Large volumes of very dilute feed, such as cell culture supernatant and clarified lysates, can be loaded onto the media without prior concentration. If the salt concentration of the sample load is lower than what is used to equilibrate the column before loading, the protein of interest can show low binding capacities, or can begin to elute from the column with unwanted contaminants.

Wash-Through

After loading of the column, follow with 2 to 3 column volumes (CV) of the high ionic strength buffer. This will wash out any unbound materials.

Elution

Elute target molecules with either a step or a linear gradient. Usually, the salt concentration at which the desired product binds is predetermined at small scale. With this knowledge, the pH and salt concentration used in wash-through are adjusted to eliminate the maximum amount of contamination before starting elution of the target.

Section 5
Scaling Up

The EconoFit Macro-Prep t-Butyl and EconoFit Macro-Prep Methyl HIC Columns are available prepacked in 1 ml formats. The resin is also available in 25 ml bottles, for scaling up methods developed using the columns. For quick scale-up, two or three columns of the same type can be connected in series, so take care to maintain an overall system pressure ≤72 psi.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 6
Regenerating, Cleaning, Sanitizing, and Storing Columns

Protein cross-contamination, frit clogging, and increased backpressure can result from running a column beyond the recommended number of uses. After repeated use, a column may run slower or produce high backpressure. We recommend that you dispose of a column after several uses. To avoid cross-contamination, designate each column for a single protein. To maintain good flow properties, clean the columns between uses.

After each run, the packed bed should be washed with 3–5 CV of a low ionic strength buffer, such as 20 mM sodium citrate or 20 mM HEPES. This is followed with 3–5 CV of water. If contamination remains, wash with 2–3 CV of 0.12 M phosphoric acid in 1% acetic acid, pH 1.5. For very difficult cleaning, follow the acid wash with a combination of nonionic detergents and then 30–70% ethanol.

Sanitization is the reduction of bioburden, that is, microorganisms and spores in the column. Before long-term storage, Macro-Prep HIC Resins should be cleaned and sanitized.

Cleaning Procedure

After elution, clean the column with 8 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5, <100 cm/hr. If not satisfactorily cleaned, continue to wash with 5 CV of 6 M guanidine HCl, <100 cm/hr. If still not clean, wash the column with 5 CV of 20% ethanol, <100 cm/hr.

Sanitization and Storage

Wash the column with 5 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5. Store the column at 4–40°C.

Section 7
Troubleshooting Guide

Problem	Possible Cause	Solution
Column clogging or slow flow rate	Particulates in sample	Filter all samples and buffers through 0.2 µm filter prior to application
No target protein in eluate	Low level of target	Check expression level of protein in starting SDS-PAGE material
	Target is not bound	Change the equilibration buffer
	Target is in flowthrough	Optimize binding conditions
	Target is not eluted	Recheck and optimize the elution buffer and conditions
Precipitation during purification	Binding capacity of column exceeded	Load less sample
	Protein aggregating	▪ Include low amount of detergent (0.1% Triton X-100, Tween 20) ▪ Include glycerol up to 10% ▪ Optimize buffer pH and salt concentration

Section 8
Ordering Information

Catalog #	Description
12009278	**EconoFit Macro-Prep t-Butyl HIC Column**, 1 x 1 ml prepacked column, 7 x 25 mm, maximum pressure 72 psi
12009277	**EconoFit Macro-Prep Methyl HIC Column**, 1 x 1 ml prepacked column, 7 x 25 mm, maximum pressure 72 psi
1580090	**Macro-Prep t-Butyl HIC Resin**, 25 ml bottle
1580080	**Macro-Prep Methyl HIC Resin**, 25 ml bottle

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