Protein Production & Assays

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Protocol

Packing of a gravity flow column for protein purification

1 GENERAL INFORMATION

Depending on the amount of protein that should be purified, gravity flow columns with different resin bed volumes are required. Since the variety of resin bed volumes of prepacked gravity flow columns is limited, sometimes it might be helpful to pack a column with a different bed volume yourself. Here, we provide a protocol that should give a guidance for correct column packing and lead to satisfactory as well as consistent purification results.

2 MATERIALS FOR PACKING OF GRAVITY FLOW COLUMNS

IBA Lifesciences offers several resins coupled with Strep-Tactin® or Strep-Tactin®XT applicable for packing of gravity flow columns and subsequent protein purification. An overview is given in the following table:

Resins for packing of gravity flow columns		Cat. No.
Strep-Tactin® Sepharose® resin	4 ml 20 ml 50 ml	2-1201-002 2-1201-010 2-1201-025
Strep-Tactin® Superflow® resin	50 ml	2-1206-025
Strep-Tactin® Superflow® high capacity resin	4 ml 20 ml 50 ml	2-1208-002 2-1208-010 2-1208-025
Strep-Tactin® Superflow® MacroPrep® resin	20 ml	2-1505-010
Strep-Tactin®XT 4Flow® resin	4 ml 20 ml 50 ml	2-5010-002 2-5010-010 2-5010-025
Strep-Tactin®XT 4Flow® high capacity resin	4 ml 20 ml 50 ml	2-5030-002 2-5030-010 2-5030-025

For packing of gravity flow columns with aforementioned resins 1x Buffer W (100 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA) is required. A tenfold concentrated buffer, 10x Buffer W (100 ml, Cat. No. 2-1003-100), is offered by IBA Lifesciences.

If empty column bodies are required, IBA Lifesciences can provide them on request with appropriate frits.

Other materials which are required for column packing but not provided by IBA Lifesciences are a spatula, a Pasteur pipette and appropriate frits (20-60 µm pore size) if column bodies from other suppliers are used.

For correct column packing, two frits are necessary: an upper frit covering the resin and a lower frit preventing the resin from escaping the column body. In addition, the upper frit ensures a uniform distribution of sample and buffer over the complete column bed. Without the upper frit, the complete binding capacity of the resin cannot be used as well as purity and yield of the purified protein can vary.

3 PROTOCOL

A video of the protocol using Strep-Tactin®XT 4Flow® as an example is available at https://www.youtube.com/watch?v=fmcX-gyw0vE .

3.1	Ensure that the column outlet is closed. If a column body without a frit at the bottom of the column bed is used, insert an appropriate frit. Empty column bodies from IBA Lifesciences already contain a frit at the bottom of the column bed.
3.2	Suspend the resin by gentle swaying.
3.3	Pipette the required amount of resin into the empty column body. For 1 ml column bed volume pipette 2 ml 50% suspension.
3.4	Remove air bubbles by gentle stirring of the resin in the column body with a spatula.
3.5	Close the column and let settle the resin for at least one hour.
3.6	In the meantime, prepare 1x Buffer W. If 10x Buffer W is available, dilute an appropriate amount by mixing one part 10x Buffer W with nine parts distilled water, e.g., 1 ml 10x Buffer W and 9 ml distilled water.
3.7	Humidify an appropriate frit with 1x Buffer W.
3.8	Carefully overlay the resin with an appropriate volume of 1x Buffer W. Prevent the resuspension of the resin. If column bodies for 1 ml column bed volume from IBA Lifesciences are used 1 ml 1x Buffer W is sufficient.
3.9	Carefully cover the resin with the wet frit. A Pasteur pipette can be helpful to gently push the frit on top of the resin. Please note that a too strong pushing compresses the resin leading to a reduced flow speed.
3.10	Store the closed column at 2-8°C until use.

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for the latest version of this manual.

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If you have any questions, please contact

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We are here to help!