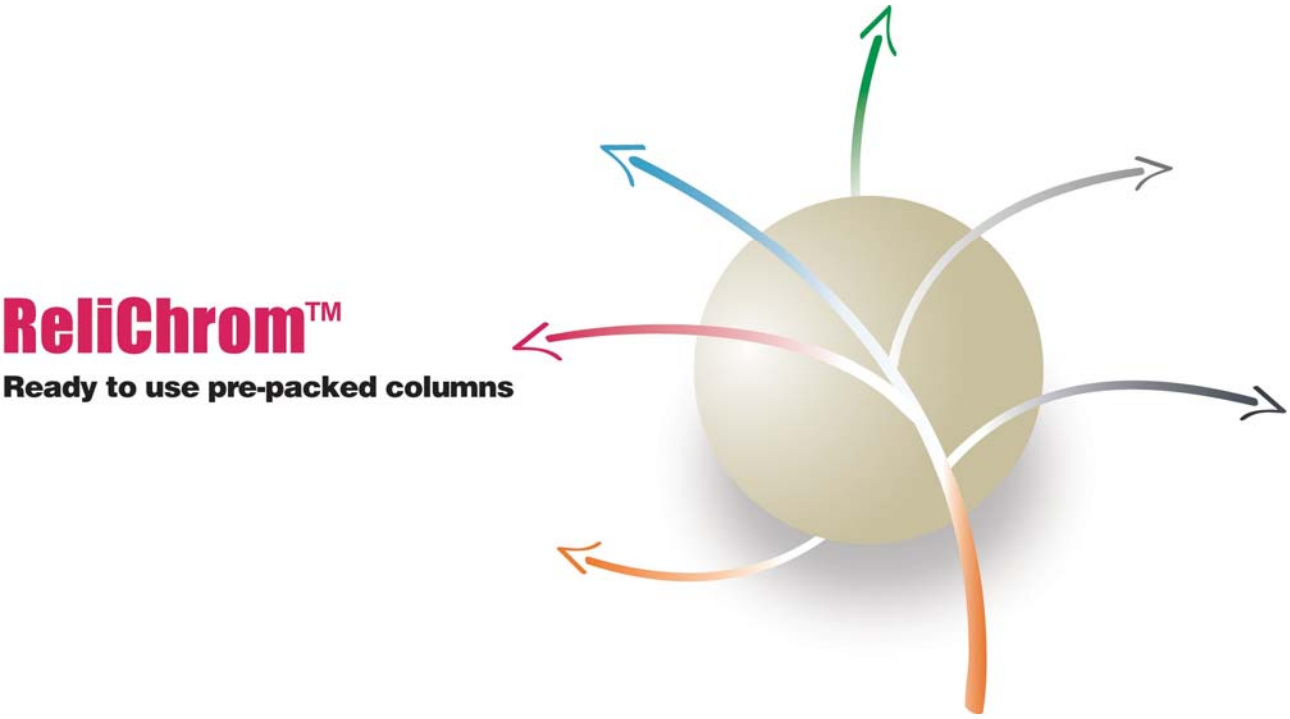
ReliChrom™

Ready to use
pre-packed columns



Resindion S.r.l.
A Subsidiary of MITSUBISHI CHEMICAL

CONTENT

Introduction

ReliChrom™ pre-packed columns have been developed by Resindion to provide a practical and reliable tool to screen **ReliSorb™SS** highly porous polymeric matrices in chromatographic bioseparations.

ReliChrom™ columns have been conceived for a possible direct connection to almost all standard LC and HPLC systems through UNF 10-32 male (1/16'' male) fittings. Each column contains 5 ml of packed resin and the resin bed height is 100 mm. These dimensions represent an excellent solution for the development of optimized purification methods and offer the possibility to increase the capacity by the connection of two columns in series.

ReliChrom™ column hardware is designed for a max operating pressure of 30 Bars (430 psi) and for a 5 - 60 °C temperature range in operation.
Overall column dimensions are 11.5 mm (8 mm ID) x 135 mm (100 mm HR) and each column is equipped with 17 µm frits.

All the components of **ReliChrom™** columns are made of polymeric materials: polypropylene (PP) and high density polyethylene (HD-PE).

ReliChrom™ columns are chemically stable in all pH range (1 - 14), in high salt concentration buffer and in common solvents (*avoid use of strong oxidizing agent and halogenated solvents*).
Proper plug-stoppers are used to grant the stability of the packed media during the storage time.

Technical Information

ReliChrom™ columns characteristics

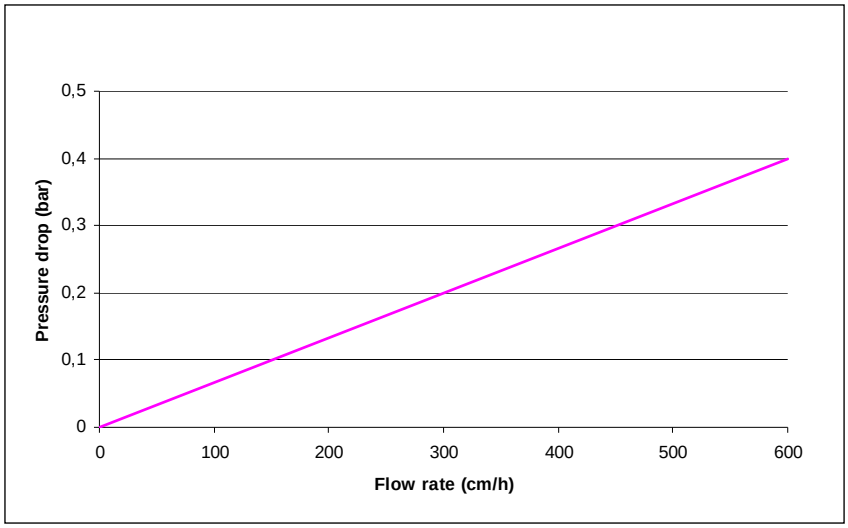
ReliSorb™ SS* packed volume:	5.0 ml
Resins bed depth:	100 mm
ReliSorb™ SS particle size range:	50 – 150 µm
Operating pH range:	1 - 14
Operating temperature range:	5 – 60 °C
Storage temperature range	5 – 30 °C (4 – 6 °C once used)

* ReliSorb™ are also available in bulk in the following grades: SS (50-150 µm), standard (75-200 µm) and EB (200-500 µm).

Pressure drop

Column dimensions:

i.d. = 0.8 cm
length = 10 cm
area = 0.5 cm^2
ReliSorb™ packed volume = 5 ml
Eluent = water
T = 25 °C



Characteristics of ReliChrom™ packed columns

NAME	FUNCTIONAL GROUP	IONIC FORM AT DELIVERY	ION EXCHANGE CAPACITY (meq/ml, min.)	BASIC APPLICATION	DYNAMIC BINDING CAPACITY (DBC)		
					BSA (mg/ml)	Lys[c] (mg/ml)	Papaine[d] (mg/ml)
CM400/SS	Carboxyl	H^+	0.15	CEX	-	min 30	-
SP400/SS	Sulphopropyl	Na^+	0.10	CEX	-	min 40	-
DA400/SS	Tertiary amine	free base	0.30	AEX	min 30[a]	-	-
QA400/SS	Quaternary ammonium	Cl^-	0.30	AEX	n.a.	-	-
IDA400/SS	Iminodiacetic	Ni^{2+}	100 (µmol/ml $^1Ni^{2+}$)	IMAC	-	-	10
BU400/SS	Butyl	-	-	HIC	min 30[b]	-	-
PH400/SS	Phenyl	-	-	HIC	min 30[b]	-	-
OD400/SS	Octadecyl	-	-	HIC	min 20[b]	-	-

[1] Ni^{2+} ionic form is applied only for ReliChrom™ pre-packed columns; different ionic forms are also available upon request.
[a] Feed solution: 10 g/l BSA in 20 mM TRIS - HCl buffer, pH 7; flow rate = 150 cm/h
[b] Feed solution: 10 g/l BSA in 20 mM phosphate buffer, pH 7 + $(NH_4)_2SO_4$ 2 M; flow rate = 150 cm/h
[c] Feed solution: 8 g/l Lysozyme in 20 mM sodium acetate buffer, pH 5; flow rate = 150 cm/h
[d] Feed solution: 20 g/l Papaine crude extract in 20 mM phosphate buffer, pH 7.2 + NaCl 200 mM; flow rate = 150 cm/h

Supply conditions

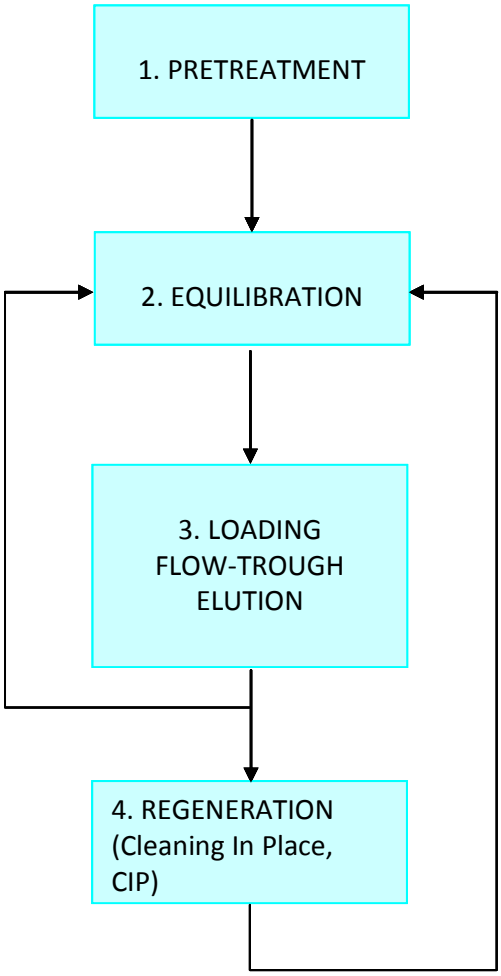
- IEX and IMAC columns: EtOH 20% aqueous solution + NaCl (final concentration 150 mM)
- HIC columns: EtOH 20% aqueous solution

Instructions for use

The flow diagram (fig. 1) shows schematically all the steps to perform a standard capture or polishing procedure on ReliChrom™ columns.

Preliminary set up:

- Rinse the chromatographic system circuit with DI water;
- After the removal of the upper stopper of the ReliChrom™ column, connect it to the chromatographic unit;
- Remove the bottom stopper of ReliChrom™ column and connect the column outlet to the specific device of the chromatographic system (Detector, fraction collector…).



Wash out the conditioning solution with 10 BV of DI water;

Start the equilibration with the desired buffer solution at an appropriate linear flow rate ;

Run the chromatographic separation, according to your individual protocol at the same flow rate as in step 2;

If necessary, perform a regeneration according to the table below.

Figure 1

Resin type	Standard Regeneration steps
AEX	▪ Regenerate with 1 ÷ 1.5 BV of NaOH 0.5 ÷ 1 M ▪ Displace the regenerant with 2 BV of DI water ▪ Only for strong AEX, condition the resin with 2 BV NaCl 0.5 ÷ 1M ▪ Rinse with 5 – 10 BV of DI water
IDA	▪ Remove residual Ni with EDTA 50 mM ▪ Displace EDTA with 2 BV of DI water ▪ Condition the resin with 1 - 1.5 BV of NaOH 0.5 M ▪ Wash the resin with 5 – 10 BV of DI water ▪ Reload Ni with a suitable salt solution ($NiCl_2$)
CEX	▪ Condition the resin with 1 BV of NaOH 0.5 M ▪ Displace the base with 2 BV of DI water ▪ Regenerate with 1 – 1.5 BV HCl 0.5 M ▪ Displace the acid with 2 BV of DI water ▪ Only for strong CEX, condition the resin with 2 BV NaCl 0.5 – 1M ▪ Rinse with 5 – 10 BV of DI water
HIC	▪ Wash the resin with 2 BV of DI water ▪ Clean the resin with 1 BV of NaOH 0.5 M in DI water or in 10-40% alcohol solution ▪ Displace NaOH solution with 2 BV of DI water ▪ Rinse with 5 BV of DI water

Remarks

- Solutions to be treated, eluents, regenerant and storage solutions should always be freshly prepared, filtered (on a 0.45 μm membrane filter) and degassed.
- Remove air bubbles from the inlet tube, before connecting the column to any liquid supply.
- Before the first use of the column and on the chromatographic system that will be used, measure, at various flow rates, the pressure drop on the **ReliChrom™** induced by the chosen eluent, in order to obtain reference data.
- Set the maximum system operation pressure to the value in which there is the max linear velocity for the minimum pressure drop.
- A significant increase of the pressure drop after repeated cycles and under the same conditions could indicate the presence of impurities: in this case the column has to be submitted to standard regeneration steps.
- After the operation and in case of long storage, the regeneration step has to be followed by a resin conditioning with proper storage solution.
- Storage temperature range: 5 – 30 °C (4 – 6 °C once used)

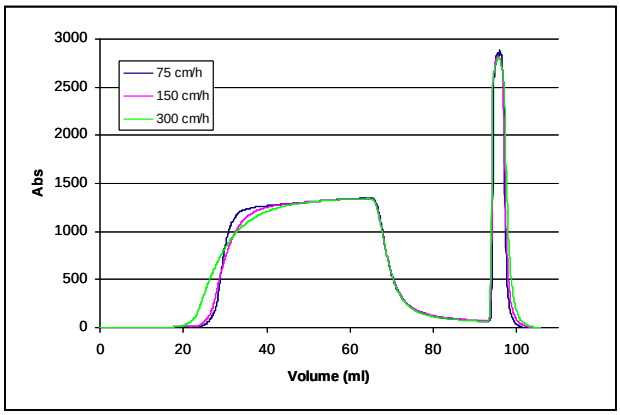
Typical Dynamic Binding Capacity (DBC)

Test: BSA capacity vs linear velocity

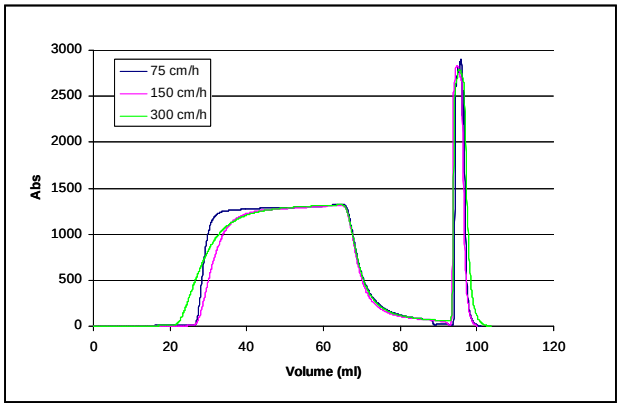
AEX

Feed solution: 10 g/l BSA in 20 mM TRIS-HCl buffer pH 7
Buffer equilibration: 6 BV of 20 mM TRIS HCl buffer pH 7
BSA loading: 12 BV
Displacement: 6 BV of 20 mM TRIS HCl buffer pH 7
Elution: 6 BV of TRIS-HCl buffer pH 7 + 4 BV of NaCl 2M

QA400/SS



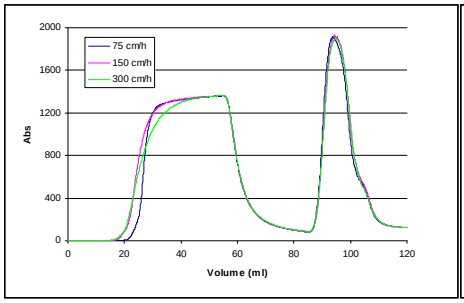
DA400/SS



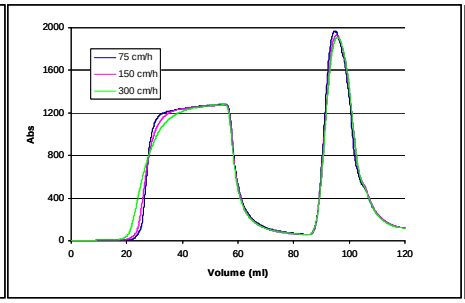
HIC

Feed solution: 10 g/l BSA in 20 mM phosphate buffer pH 7 + $(NH_4)_2SO_4$ 2M
Buffer equilibration: 6 BV of 20 mM phosphate buffer pH 7 + $(NH_4)_2SO_4$ 2M
BSA loading: 10 BV
Displacement: 6 BV of 20 mM phosphate buffer pH 7 + $(NH_4)_2SO_4$ 2M
Elution: 4 BV of 20 mM phosphate buffer pH 7

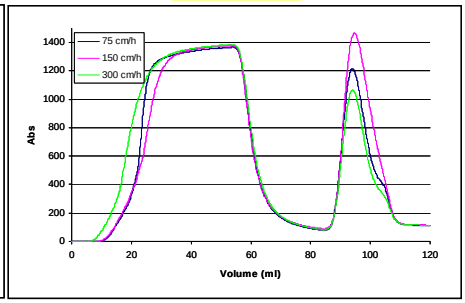
BU400/SS



PH400/SS



OD400/SS

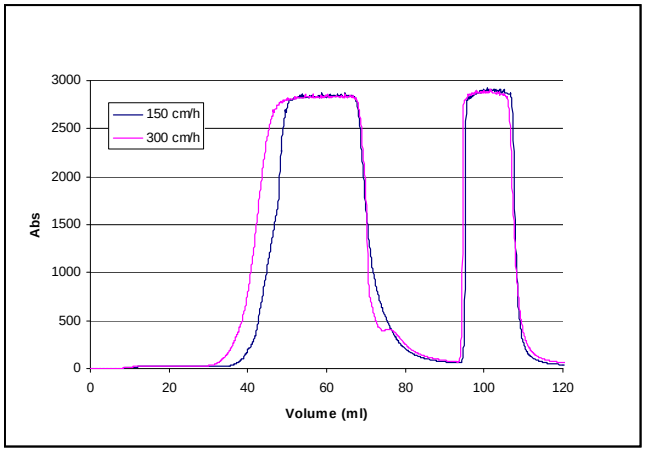


CEX

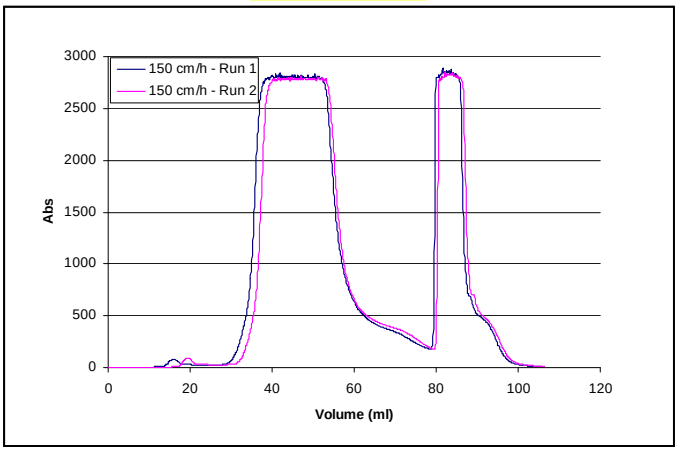
Test: lysozyme capacity vs linear velocity

Feed solution: 8 g/l Lysozyme in 20 mM sodium acetate buffer, pH 5
Buffer equilibration: 6 BV sodium acetate buffer 20 mM, pH 5
Displacement: 6 BV sodium acetate buffer 20 mM, pH 5
Elution: 6 BV sodium acetate buffer 20 mM, pH 5 + NaCl 1M

SP400/SS



CM400/SS

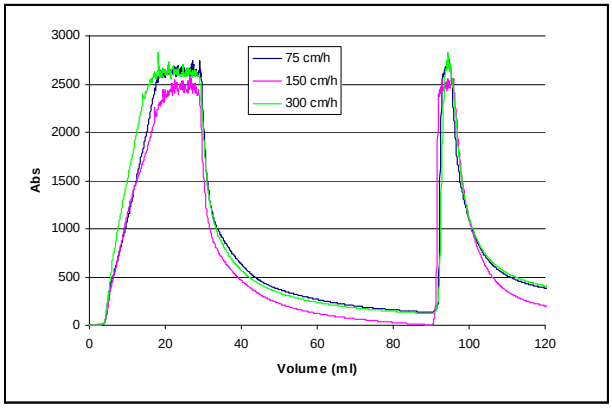


IMAC

Test: papain capacity vs linear velocity

Feed solution: 20 g/l Papain crude extract in 20 mM phosphate buffer, pH 7.2 + NaCl 200 mM
Buffer equilibration: 12 BV phosphate buffer 20 mM, pH 7.2 + NaCl 200 mM
Displacement: 8 BV phosphate buffer 20 mM, pH 7.2 + imidazole 0.5 M
Elution: 4 BV phosphate buffer 20 mM, pH 7.2 + NaCl 200 mM

IDA400/SS



Purchase information

Product Description	Code	Quantity
ReliChrom™ CM400/SS	011748	1 x 5 ml
ReliChrom™ SP400/SS	013748	1 x 5 ml
ReliChrom™ DA400/SS	023748	1 x 5 ml
ReliChrom™ QA400/SS	024748	1 x 5 ml
ReliChrom™ BU400/SS	032748	1 x 5 ml
ReliChrom™ PH400/SS	034748	1 x 5 ml
ReliChrom™ OD400/SS	033749	1 x 5 ml
ReliChrom™ IDA400/SS	012748	1 x 5 ml
UNF 10-32 (1/16'') male fittings	041747	Upon request

ReliChrom™ Material Support File

Resindion S.R.L. is an UNI EN ISO 9001:2008 certified Company. Technical and basic regulatory documents, supplied with each ReliChrom™ column, are related to the Handling Instructions and Column Performance Report (HICPR). Upon request Resindion S.R.L. can supply the information related to ReliSorb™ packed resins (i.e. Certificate of Analysis, while PTDS and MSDS can be directly downloaded from the web site).

Technical assistance

Resindion S.R.L. qualified technical and marketing team guarantees all the necessary customer assistance on ReliChrom™ selection and application. Please consult Resindion at: technicalservice@resindion.com.

Resindion S.r.l.
A Subsidiary of MITSUBISHI CHEMICAL

Resindion S.R.L.
Via Roma 55
20082 Binasco (MI), Italy
Phone: +39-02-9001301
Fax: +39-02-90090774
E-mail: info@resindion.com
Web site: www.resindion.com

Mitsubishi Chemical Corporation
Palace Building
1-1 Marunouchi 1-chome, Chiyoda-ku
Tokyo 100-8251, Japan
Phone: +81-3-6748-7146
Fax: +81-3-3286-1209
Web site: www.diaion.com