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# GLYCANPAC AXH-1 COLUMN

***Quick Start***

## 1. Overview

The Thermo Scientific™ GlycanPac AXH-1 column is a high-performance, silica-based HPLC column for simultaneous separation of glycans by charge, size and polarity. It is designed for high-resolution and high-throughput analysis with unique selectivity for biologically important glycans, either labeled or un-labeled, by LC-fluorescence and LC-MS methods.

## 2. Main features

- Unique glycan selectivity based on charge, size and polarity.
- Excellent resolution for both unlabeled and labeled glycans.
- Useful for both high-resolution glycan profile characterization and easy quantification of glycans based on charge.
- Compatible with fluorescence and MS detection methods.
- High chromatographic efficiency and excellent column stability.

## 3. Physical data (Table 1)

| | GlycanPac AXH-1 Column (3 µm) | GlycanPac AXH-1 Column (1.9 µm) |
|---|---|---|
| **Column chemistry** | WAX and HILIC Mixed-Mode | WAX and HILIC Mixed-Mode |
| **Silica substrate** | Spherical, high-purity, porous | Spherical, high-purity, porous |
| **Particle size** | 3 µm | 1.9 µm |
| **Surface area** | 300 $m^2$/g | 220 $m^2$/g |
| **Pore size** | 120 Å | 175 Å |

## 4. Specifications and Recommended Operational Parameters (Table 2)

| Column Particle size | Column Dimension | P/N | Maximum Pressure (psi) | pH Range | Temperature Limit (ºC) | Solvent/Aqueous Compatibility | Recommended Flow Rate (mL/min) | Maximum Flow Rate (mL/min) |
|---|---|---|---|---|---|---|---|---|
| 1.9 µm | 2.1 x 100 mm | 082473 | 7,000 | 2.0 – 8.0 | < 60 | Compatible with 0 – 90% aqueous and common HPLC solvents (except acetone) | 0.1 - 0.4 | 0.50 |
| | 2.1 x 150 mm | 082472 | 10,000 | 2.0 – 8.0 | < 60 | | 0.1 - 0.4 | 0.50 |
| | 2.1 x 250 mm | 082471 | 15,000 | 2.0 – 8.0 | < 60 | | 0.1 – 0.4 | 0.50 |
| 3 µm | 4.6 x 150mm | 082468 | 6,000 | 2.0 – 8.0 | < 60 | | 0.6 – 1.2 | 1.50 |
| | 3,0 x 150 mm | 082469 | 6,000 | 2.0 – 8.0 | < 60 | | 0.3 – 0.6 | 0.75 |
| | 2.1 x 150 mm | 082470 | 6,000 | 2.0 – 8.0 | < 60 | | 0.1 – 0.4 | 0.50 |

## 5. Operational Guidelines

- **All new columns** or any column not in use for longer than 3 days **must** be treated using the cleaning procedure described below in Table 3 before use. Equilibriate the column and perform 3 blank injection prior to analysis of real samples.

**NOTE**

***Buffered solutions must be used for analysis and storage.***

- Operate the column within operating specifications (see Table 2 for details).
- Avoid sudden pressure surge.
- Follow the direction of flow is marked on the column.
- Always use guard columns when analyzing samples and replace them before exhausted.
- Column storage: use mobile phase for short-term storage (< 24 hours) and a solution containing 90% acetonitrile and 10% ammonium formate buffer (e.g. 100 mM, pH4.4) for long-term storage (> 24 hours),
- Mobile phase: acetonitrile/ammonium formate buffer (e.g. 100 mM, pH4.4) system is recommended for both LC/fluorescence and LC/MS applications
- Whenever abnormal peak broading and/or peak tailing is observed, perform the column cleaning described below.

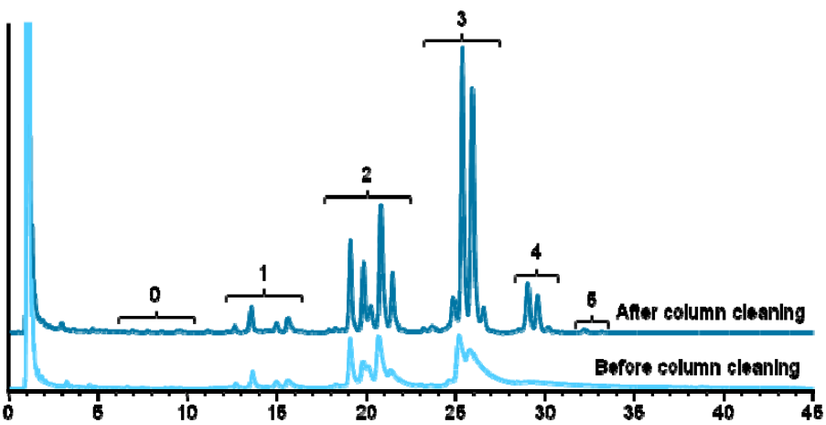
NOTE

***Column performance is affected by the contaminants from samples, LC system, and mobile phase. Metal contamination can be witnessed quite often because most LC system and column hardware are made from stainless steel which will bleed out iron over time. When this happens, perform the column cleaning e described below (also in Section 4.7 in the GlycanPac AXH-1 Column Manual) to restore the performance of the column.***

## 6. Cleaning Procedure (Table 3)

| Time (min) | Acetonitrile (%) | 50 mM sodium pyrophosphate in 100 mM ammonium formate, pH 4.4 (%) | D.I. water (%) | 100 mM Ammonium formate buffer, pH 4.4 (%) | Flow (mL/min) 2.1 mm dia. column | 3.0 mm dia. column | 4.6 mm dia. column |
|---|---|---|---|---|---|---|---|
| 0 | 50 | 0 | 0 | 50 | 0.25 | 0.51 | 1.20 |
| 5 | 20 | 0 | 0 | 80 | 0.25 | 0.51 | 1.20 |
| 10 | 20 | 80 | 0 | 0 | 0.25 | 0.51 | 1.20 |
| 35 | 20 | 80 | 0 | 0 | 0.25 | 0.51 | 1.20 |
| 36 | 20 | 0 | 0 | 80 | 0.25 | 0.51 | 1.20 |
| 50 | 20 | 0 | 0 | 80 | 0.25 | 0.51 | 1.20 |
| 60 | 80 | 0 | 0 | 20 | 0.25 | 0.51 | 1.20 |
| 61 | 78 | 0 | 20 | 2 | 0.25 | 0.51 | 1.20 |
| 70 | 78 | 0 | 20 | 2 | 0.25 | 0.51 | 1.20 |

These conditions are appropriate for all 100 or 150 mm long columns. Increase the times by 60% for the 250 mm long column.




Performance comparison before and after cleaning of the column for 2-AB labeled glycans from bovine fetuin. Peaks are grouped by sialylation number.

## 7. Ordering Information (Table 4)

| | Particle Size | Column Dimensions | P/N | Required Holder |
|---|---|---|---|---|
| Analytical | 1.9 µm | 2.1x250 mm | 082521 | |
| | | 2.1x150 mm | 082472 | |
| | | 2.1x100 mm | 082473 | |
| | 3µm | 4.6x150mm | 082468 | |
| | | 3.0x150 mm | 082469 | |
| | | 2.1 x 150 mm | 082470 | |
| Guard pkg. of 2 | 3µm | 4.6 x 10 mm | 082474 | P/N 069580 |
| | | 3.0 x 10 mm | 082475 | P/N 069580 |
| | | 2.1 x 10 mm | 082476 | P/N 069580 |