

Glycap-3A™

NOTE!

For research use only, not for human use, not for commercial use, not for clinical diagnostic use. Patents US10221210B2, EP2734537A1

Glycap-3A (human Fc gamma RIIIA) pre-packed columns can separate non-fucosylated, bisected GlcNAc, IgG1, and IgG3 from complex antibody mixtures of monoclonal or polyclonal IgG. The columns, available in 1 mL and 5 mL sizes, are packed with an affinity resin to which human Fc gamma RIIIA (CD16A) is immobilized.

Glycap-3A is operated by loading a neutral pH and eluting high affinity glycoforms and subclasses at low pH (for example, pH 3-4.5).

Purification

The parameters below are exemplary of typical operating conditions and are not limiting to acceptable conditions, methods or resin capabilities¹.

- 1. Equilibrate the column with ≥5 column volumes (CV) buffer at neutral pH 7-7.5.
- Load the sample at neutral pH, possible from pH 5-8.5, if load IgG is 10% afucosylated then load to 10x the
 resin capacity to achieve high enrichment of afucosylated IgG. Dynamic binding capacity is ~2 mg/ml of
 afucosylated IgG.
- 3. Wash with 5-10 CVs buffer at neutral pH.
- 4. Elute at pH 3.0-4.2 with elution buffers like glycine or citrate, pH 3.2 or higher is a good place to start if antibody is pH sensitive. Asymmetric afucosylated antibodies elute at a higher pH (for example pH 4.2) than symmetrically afucosylated antibodies (for example pH 3.0). The afucosylated IgG enrichment factor is protein specific. Gradient elutions speciate glycoforms.

To prevent resin compression, pressure drop across the column should not exceed 30 psi. Ideally operate at 3-minute residence time or greater.

Strip

≥5 CV 100 mM glycine or citrate pH 3.0-4.2 with 0-1000 mM MgCl2. The resin is not NaOH stable.

Storage

Store the resin at 2 to 8°C in 20% ethanol at neutral pH.

Product description

	Glycap 3A 1 mL column	Glycap 3A 5 mL column
Dynamic binding capacity (DBC)	~ 2 mg afucosylated lgG/ml resin²	~ 2 mg afucosylated lgG/ml resin ²
Operating flow rate	<1mL/min	< 4.7 mL/min
Pressure rating (column)	1200 psi	600 psi
pH stability	5 - 8.5	5 – 8.5
Operating temperature	2 to 25 ∘C	2 to 25 ∘C
Storage	2 to 8°C in 20% ethanol at neutral pH^3	2 to 8°C in 20% ethanol at neutral pH³

^{2.} Dynamic binding capacity determined at ≥3-minutes residence time. 3. Column supplied in PBS + 20% ethanol.

Ordering information

This product is distributed by Bio-Works <u>www.bio-works.com.</u>