S sterogene bioseparations

WGA Superflow 4

Instructions for Use

WGA Superflow 4

Wheat Germ Agglutinin (WGA) Superflow 4 is an affinity chromatography resin used for the purification of glycoproteins containing N-acetyl glucosamine (GlcNAc) groups or sialic acids. The immobilized ligand is a highly purified WGA lectin isolated from wheat germ (*Triticum vulgaris*). The resin is specifically used to purify insulin receptors, several serum and surface glycoproteins. WGA Superflow 4 is a highly stable resin and shows no leaching with commonly used buffers and in a pH range of 3-13. The resin can be used multiple times without the loss in yield and ligand activity.

WGA is a homodimeric lectin and has specificity for terminal GlcNAc and *N*-Acetylneuraminic acid (sialic acid) residues on glycoproteins. It preferentially binds to the dimeric or trimeric form of GlcNAc.

WGA Superflow 4 resin is supplied in as 50% slurry in PBS containing 20 Mm GlcNAc and 0.05% Sodium azide.

Upon receiving, store the resin at 2-8 °C. Do not freeze.

Key features:

Matrix support: 4% crosslinked agarose beads

Immobilized ligand: Highly purified Wheat Germ Agglutinin (WGA) lectin

Ligand density: 5 mg lectin/ml of agarose beads

Binding capacity: 3-5 mg of target protein/ml of resin

Storage buffer: PBS with 0.05% sodium azide.

Elution buffer: 0.5 M N-Acetyl-D-Glucosamine or Chitin Hydrolysate in PBS

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Protocol:

Purification of GlcNAc/sialic acid containing glycoproteins from serum

WGA Superflow 4 resin can be packed in a disposable column (1-5 ml) and can also be used in a spin column depending on the scale of purification. Dilute the serum 1:1 in equilibration buffer (PBS).

- 1. Remove the resin from the fridge and equilibrate it at room temperature.
- 2. Pack the required amount of slurry in the column.
- 3. Equilibrate the packed column with 5 column volumes of PBS.
- 4. Apply the diluted serum on the column with a slow flow rate.
- 5. Wash the column with 5-10 bed volumes of PBS at high flow rate (until no protein is detected in the flowthrough, based on OD_{280}).
- 6. Elute the bound glycoproteins with 0.5 M solution of N-Actyl-D-Glucosamine or chitin hydrolysate in PBS at slow flow rate and in multiple small fractions. If necessary, the pH of the elution buffer can be adjusted to pH 3.0 by acetic acid.
- 7. Measure the OD₂₈₀ to determine the concentrated fractions. Run the SDS-PAGE and pool the fractions, dialyze in required buffer, concentrate, and store at required temperature.
- 8. The WGA Superflow 4 resin can be regenerated by washing the column with more than 20 column volumes of PBS. The clean column is then ready for another round of purification or can be stored at 4 °C in buffer containing 0.05% of sodium azide.

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