SUPPLEMENTAL PROTOCOL

WGA, Agarose: Use & Regeneration

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Before initial use, wash gel with 10 column volumes of buffer without any stabilizing sugar. Many buffers may be employed such as that in the suspension solution listed on the product specifications. The sodium azide is not required for use, but is recommended for storage.

After sample application, wash column with 2–3 column volumes of buffer (or until $OD_{280\ nm}$ is reduced to a satisfactory level) before elution. Use 0.5 M N-Acetyl-D-Glucosamine in buffer for elution of glycoconjugates which bind to this immobilized lectin. For those glycoconjugates having a very high affinity for WGA, it may be necessary to lower the pH of the eluting sugar solution to pH 3.0 with acetic acid.

After elution, the column can be prepared for reuse by washing with 10 column volumes of buffer. The column can be prepared for storage by equilibrating the column with buffer containing 0.08% sodium azide and, if possible, 20 mM, GlcNAc, which will stabilize the lectin.

The gel can be used many times in this manner. With each use, it is possible that impurities may bind nonspecifically to the column. The amount of impurities depends on the condition of the samples being applied. For this reason, a separate procedure can be employed to remove these impurities. These impurities may increase the volume required for elution washes or may reduce the binding capacity of the gel.

One method of regeneration involves washing the column with 5 column volumes of buffer, pH = 3.0 (using acetic acid) with 1 M NaCl. After this wash, the column should be rinsed with 10 column volumes of buffer. It is then ready for use. Alternative regeneration methods may also be effective.

It is typical to expect a 3–5% loss in capacity per use.

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