Chitin Beads

1-800-632-7799
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www.neb.com

S6651S 012120315031

S6651S

20 ml **Lot: 0121203**

Store at 4°C **Exp: 3/15**

Description: An affinity matrix for the isolation of target proteins fused to an intein-chitin binding domain fusion (1).

Source: A pure chitin bead supplied as a 40 ml slurry in 20% ethanol. The column is poured and after washing with five column volumes of buffer it is ready for use.

Binding Capacity: 2.0 mg maltose-binding protein/ml bed volume released from the bead after cleavage of the fusion protein expressed from pMYB5.

Quantitative Analysis: Crude extract from *E. coli* containing a plasmid expressing a maltose-binding protein-intein-chitin binding domain fusion protein is passed over a 1 ml column at 4°C. The column is washed with > 10 column volumes of 20 mM HEPES (pH 8.0) 500 mM NaCl, 0.1 mM EDTA, 0.1% Triton-X100. The column is then quickly flushed with 3 column volumes of 30 mM DTT (freshly diluted in cleavage buffer [20 mM HEPES (pH 8.0), 500 mM NaCl, 0.1 mM EDTA]. The flow to the column is stopped, and the column is left at 4°C overnight. MBP is eluted using 3 column volumes of cleavage buffer without DTT.

Regeneration: The packed chitin bead column must be stripped of both the fusion partner (intein-CBD) and the uncleaved fusion protein. Wash the column with 3 bed volumes of the 0.3 M NaOH stripping solution. Allow resin to soak 30 minutes, wash the resin with an additional 7 bed volumes of stripping solution. Wash with 20 bed volumes of water, followed by 5 bed volumes of column buffer.

Notes: We recommend using gravity flow to run the column. The column should have a wide diameter.

The column may be regenerated 5 times.

Store at 4°C. The used resin should be stored in column buffer. For long term storage add 0.02% sodium azide to the column buffer.

References:

1. Chong, S., Mersha, F. B., Comb, D. G., Scott, M. E., Landry, D., Vence, L. M., Perler, F. B., Benner, J., Kucera, R. B., Hirvonen, C. A., Pelletier, J. J., Paulus, H., and Xu, M.-Q. (1997). Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. Gene 192, 271-281.

Average Elemental Analysis of Chitin Beads:

Element	Chitin Bead	%Theoretical
C	42.7%	46.7%
O	40.7%	41.5%
H	7.0%	6.4%
N	6.2%	5.6%

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CERTIFICATE OF ANALYSIS

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