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ProductInformation

Maltoheptaose Agarose
Product Code **M 9676**
Storage Temperature 2–8 °C

Product Description

Maltoheptaose agarose is an affinity chromatography matrix designed for purification of molecules such as maltose binding protein (MBP) that bind to maltose and related sugars. This resin has been designed so that it is similar to amylose resin, but with lower molecular weight glucose polymers resulting in a larger number of potential binding sites which, in turn, gives the resin a higher protein capacity.[1,2]

This resin will bind a minimum of 6 mg of maltose binding protein per ml of packed resin.

Preparation Instructions

This product is prepared as a 50% suspension in a 30% aqueous ethanol solution and is sold as ml of packed gel. The ethanol must be removed just prior to use. Thoroughly resuspend the resin with gentle inversion and remove an appropriate aliquot for use. Pour the resin into a clean chromatography column using standard techniques, or handle the resin batch-wise, depending on the scale of purification planned. Allow the storage solution to drain completely from the resin in a column (when using a chromatography column), or on a filter (when using a batch-wise method). Next, wash the resin with a minimum of 3 bed volumes of water and equilibrate by washing with 3 bed volumes of an appropriate equilibration buffer. A typical equilibration buffer consists of 25 mM MOPS, pH 7.4, with 0.2 M sodium chloride and 1 mM EDTA. Remove most of the equilibration buffer before use and do not allow the resin to remain in column buffer for extended periods of time (> 24 hours).

Storage

When not in use, store the maltoheptaose agarose at 2–8 °C in either 30% ethanol or in buffer with another suitable antimicrobial agent such as 0.1% sodium azide.

Amylases have the potential to degrade the ligand. Loss of the ligand will reduce the capacity of the resin with time. It is recommended that the resin be cleaned immediately after use with 3 column volumes of water, followed by 3 volumes of 0.1% SDS in water, and finally 3 volumes of water. After cleaning, the resin should placed in a suitable storage solution (30% ethanol or a buffer containing 0.1% sodium azide).

Procedure

The exact procedure should be empirically determined by the end user, since conditions may vary for different proteins and different extraction procedures. There are many variations in conditions that can be used to purify a maltose binding protein. The pH can range from 7-8, and buffers such as Tris-HCl and phosphate can be used. Sodium chloride or potassium chloride of up to 2.0 M can be used to prevent non-specific binding to the resin.

Detergents such as TRITON™ X-100 and TWEEN® 20 will prevent binding or severely decrease the capacity of the resin. Denaturants such as urea and guanidine HCl will also prevent binding.

A general procedure for purifying maltose binding protein from crude *E. coli* extracts is provided.

1. The cells (approximately 1 liter) are grown under conditions that induce production of maltose binding protein.

2. The cells are harvested by centrifugation at 5,000 x *g* for 30 minutes at 4 °C.

3. The *E. coli* cells are resuspended in a binding buffer (25 mM MOPS, pH 7.4, with 0.2 M sodium chloride and 1 mM EDTA). Use approximately 15 ml of buffer per gram of wet cell weight. The volume of buffer should not exceed 50 ml. If larger amounts of cells are required, they can be processed in multiple 50 ml batches.

4. The resuspended cells are frozen in a dry ice/ethanol bath or at –20 °C in a freezer. Cell lysis is enhanced during the slow freezing.

5. The container with the cells is then thawed in a container of room temperature water. The cell suspension should be thick and viscous.

6. The cells are then placed in an ice bath and are sonicated for about 2 minutes to reduce viscosity.

7. The cell debris is removed by centrifuging for 30 minutes at 10,000 x *g*.

8. After centrifugation, decant the supernatant into a fresh container and discard the cell pellet. If the pellet is soft and a portion of the pellet decants into the supernatant pool, centrifuge the supernatant again to remove the residual cell debris. An alternate method of extracting the maltose binding protein is the osmotic shock method.[3] This method is slightly more time consuming and releases only those proteins found in the periplasmic space.

9. Filter the extract through a 0.45 µm filter to remove any remaining whole cells.

10. Dilute the filtered extract with 4 volumes of binding buffer (25 mM MOPS, pH 7.4, with 0.2 M sodium chloride and 1 mM EDTA). The protein concentration should be about 2-6 mg/ml.

11. An appropriate amount of resin should be equilibrated in a column as described in the preparation section. The amount of resin necessary depends upon the amount of maltose binding protein in the extract. The amount of MBP in the extract should not be more than the maximum capacity of the resin in the column; otherwise MBP may leak through the column.

12. The crude extract is loaded onto the column at a flow rate of approximately 5-10 column volumes per hour. If this rate is exceeded, some of the MBP may leak through the resin.

    The extract should be loaded immediately after preparation and the loading time should not be excessively long (> 6 hours). If it will take a considerable amount of time to load the extract using a column format, then a batch absorption process should be used. Batch absorption uses resin that has been equilibrated and mixes the resin directly into the protein solution to capture the protein of interest. The resin should be drained and then added to the extract to bind the MBP protein. It should be gently mixed with glass rod for 10-20 minutes to bind the target protein (do not use a magnetic stir bar since this will destroy the resin beads). After the material has been bound, the extract is filtered off and the resin is washed with fresh buffer. The resin can then be placed in a column and washed thoroughly and eluted as described for the column.

13. After all of the extract is loaded, the column is washed with fresh binding buffer. The flow rate during the washing step can be increased to 10-20 column volumes per hour. The column should be extensively washed until the A_{280} of the material eluting from the column is <0.05.

14. The MBP is eluted from the column using 10 column volumes of elution buffer (25 mM MOPS, pH 7.4, with 0.2 M sodium chloride, 1 mM EDTA, and 10 mM maltose). Fractions should be taken from the elution of the column. The fraction size should be about 1/4 the column volume and the flow rate should be 10 column volumes per hour. The MBP should begin to elute after the first column volume of elution buffer has passed through the column (fraction 4 or 5). The eluted fractions should be assayed by A_{280} and/or SDS-PAGE to determine which are suitable.

References

1. Ferenci, T., and Klotz, O.K., FEBS Lett., **94**, 213-217 (1987).
2. Kellerman, O.K., and Ferenci, T., Meth. Enzym., **75**, 459-463 (1982).
3. Nossal, N.G., and Heppel, L.A., J. Biol. Chem., **241**, 3055-3062 (1966).