Product Information

Product Name:	Dextrin Beads 6FF
Product Code:	SA026025/SA026100
Size(s):	25ml & 100ml

Product Description:

Dextrin Beads 6FF is an affinity resin used in the purification of recombinant proteins tagged with maltose binding protein (MBP-tagged protein). Fusion of recombinant proteins with affinity MBP-tags increases the level of expression and solubility of the target proteins.

The robust and highly cross-linked 6% agarose base matrix has been optimized to provide high binding specificity, in addition to the high physical and chemical stability of the matrix. Affinity purification of recombinant proteins is performed under physiological conditions, alongside with mild elution by maltose, to preserve the activity in target proteins. Mild conditions may allow for purification of intact protein complexes.

Product Specification:

Chromatography Technique:	Tagged protein affinity purification
Matrix:	Highly cross-linked 6% agarose
Ligand:	Dextrin
Binding Capacity:	>10mg MBP-tagged protein/ml medium
Particle Size:	45 - 165μm
Maximum Pressure:	3bar (0.3MPa, 43psi)
pH Stability:	pH 3 - 12
Storage Conditions	2 – 8°C, 1XPBS with 20% ethanol (Do not freeze)

Test Items	Specifications
Appearance	White or white-like beads
Volume	50% suspension
Binding Capacity	>10mg MBP-tagged protein/ml medium

Operation Protocol

1. Buffer Preparation

Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22μm or 0.45μm filter before usage.

Binding/Wash Buffer: 20mM Tris-HCl, 200mM NaCl, 1mM EDTA, pH7.4
Elution Buffer: 20mM Tris-HCl, 200mM NaCl, 1mM EDTA, 10mM Maltose, pH 7.4
Note: 1mM DTT or 10mM β-mercaptoethanol can be added

2. Sample Preparation

2.1. Harvest cells from an appropriate volume of bacterial culture by centrifuging at 7,000 rpm for 10-15 minutes at 4°C. Discard the supernatant
2.2. Resuspend the cell pellet in 1:10 ratio (w/v) with Binding/Wash buffer, either through pipetting or inverting at room temperature of 20-25°C or 4°C
2.3. Disrupt the resuspended cells by sonicating in short bursts
2.4. Centrifuge the lysed cells at 12000 × g for 40 minutes at 4°C. Transfer the supernatant to a new tube. To determine the solubility of the protein, reserve a fraction of the supernatant and pellet for SDS-PAGE analysis

3. Column Packing

3.1. Flush the end-piece and adaptor with Binding Buffer to remove the air from the column dead spaces. Ensure that there is no air trapped under the column net
3.2. Close the column outlet, while leaving the column net covered with Binding Buffer
3.3. Shake the beads container to resuspend the stored beads (Avoid stirring the sedimented medium). To minimise the introduction of air bubbles, pour the slurry down a glass rod while holding it against the column wall.
Note:
If a packing reservoir is in use, fill the remaining column and reservoir with Packing Buffer immediately. Mount the adaptor/lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adaptor or in the inlet tubing.
3.4. Open the bottom outlet of the column and set the pump to run at the desired flow rate. The Dextrin Beads 6FF is packed at a constant pressure of approximately 1bar (0.1MPa).
    i. If the packing equipment does not include a pressure gauge, a packing flow rate of approximately 400cm/h (10cm bed height, 25°C, low viscosity buffer) should be used
    ii. If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver to provide a reasonably wellpacked bed. **DO NOT** exceed 75% of the packing flow velocity in subsequent chromatographic procedures
3.5. When the bed is stabilized, close the bottom outlet and stop the pump.
Note:
    i. If a packing reservoir is in use, disconnect the reservoir and fit the adaptor to the column.
    ii. If the column is in use, carefully place the top filter on top of the bed before fitting the adaptor to the column
3.6. With the adaptor inlet disconnected, push the adaptor (approximately 2mm) down into the bed. This allows the packing solution to flush the adaptor inlet.
3.7. Connect the pump and open the bottom outlet, before resuming packing. The bed should be further compressed and a space will be formed between the bed surface and the adaptor.
3.8. Close the bottom outlet. Disconnect the column inlet and lower the adaptor (approximately 2mm) into the bed. Connect the pump. The column is now ready for use

4. Column Purification Protocol

4.1. Fill the syringe/pump tubing with Binding Buffer. Remove the stopper and connect the column to the syringe/pump tubing (using the connector provided) in a “drop to drop” motion, to avoid introduction of air into the column. Remove the snap-off end at the column outlet
4.2. Wash the column with 10 column volumes of Binding Buffer
4.3. Apply the sample, either through the syringe fitted to the connecter or pumping it into the column
4.4. Wash the column with 5-10 column volumes of Binding Buffer, until there is an absence of materials in the effluent
4.5. Elute with 5 column volumes of Elution Buffer (Volumes of Elution Buffer varies with the ligand-protein interaction).

5. Results Analysis

The eluted fractions can be identified using UV absorbance, SDS-PAGE and western blot

6. Cleaning-in-Place

Dextrin Beads 6FF can be reused several times. If precipitation and protein aggregation occurs, it can cause the loss of flow velocity and combined loads. The medium would then have to be cleaned as follows:

i. Wash the column with 3 column volumes of deionized water
ii. Wash the column with 3 column volumes of 0.1% SDS or 0.5M NaOH
iii. Wash the column with 3 column volumes of deionized water again
iv. Store at 2 - 8°C with 20% ethanol. Re-equilibrate the column with 10 column volumes of Binding Buffer before starting the next purification process

Storage

For long-term storage, the Dextrin Beads 6FF should be stored in 20% ethanol at 2 - 8°C.

Troubleshooting Guide

Problems	Possible Causes	Solutions
The back pressure is too high	The column is clogged	Cleaning-in-Place (refer to **section 6**)
	The sample solution contains precipitate	Filter the sample solutions through a 0.22 μm or 0.45 μm filter
The eluted fraction is not pure	Protein degradation	Addition of appropriate protease inhibitors (or inhibitor cocktails), such as PMSF and EDTA, to the lysis solution and wash solution
	Inadequate washing of the column	Increase the volume of Wash Buffer
There is no binding	Expression of target protein is very low	• Check the protein expression level by estimating the amount in the extract, flow through, eluted fraction and pellet upon centrifugation

		• Apply large sample volumes
	There are some interference factors in the sample or buffer	Sample dialysis or diluted the sample with Binding Buffer
	Production of amylase by cells could have affected the proteins and the medium	Inhibit the expression of amylase by adding glucose to the culture medium
	The contact time is too short	The sample and the medium can be incubated at room temperature (20–25°C) for ≥ 2 hours