MBP-Tag Protein Purification Kit (Dextrin)

Cat #: orb1173392 (manual)

Size: 1 mL/1 mL×5

MBP-Tag Protein Purification Kit (Dextrin), soluble protein purification optimized

Product name: MBP-Tag Protein Purification Kit (Dextrin)

Catalog number: orb1173392

Capacity: >20 mg MBP-tagged protein/mL

Bead size: 45-165 μm

Storage: Stable for 12 months at 4℃ from date of shipment

Note: Storing according to the recommended storage conditions after the package is opened

Assay Principle

Maltose binding Protein (MBP) is a member of the maltose/maltodextrin system of E.coli which is accountable for the uptake and efficient catabolism of maltodextrins. MBP-Tag is very effective in improving the expression level and solubility of many proteins as a fusion protein. MBP-Tag Protein Purification Kit provides a simple, rapid, and efficient purification of MBP-Tag proteins.

Materials Supplied and Storage Conditions

Kit components	Size		Storage condition
	1 mL	1 mL×5	
MBP-Tag Purification Nickel Column	1 mL	1 mL×5	4℃
Binding/Wash buffer (10×)	30 mL	100 mL+50 mL	4℃
Elution buffer (10×)	15 mL	75 mL	4℃

Materials Required but Not Supplied

- 0.22 μm or 0.45 μm filter
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Various glassware for preparing reagents and buffer solutions

Reagent Preparation

It is recommended to filter all water before use by passing through a 0.22 μm or 0.45 μm filter. For most proteins, the following buffer are recommended:

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Reagent	Volume		
	Binding/Wash buffer (10×)	Elution buffer (10×)	Water (mL)
Binding/Wash buffer	6	0	54
Elution buffer	0	3	27

Note: 1 mM DTT or 10 mM β-mercaptoethanol can be included in the Binding and Elution Buffer.

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22 μm or 0.45 μm filter before it is applied to the medium to prevent clogging the column. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column. Be careful not to exceed the resin's binding capacity.

Procedure for Sample Purification

1. Fix Column. Move the top and bottom stopper, and let the storage buffer drain away.
2. Add 2 resin-bed volume Binding buffer to the column. Equilibrate the column, and drain away the Binding buffer. Repeat this step for three times.
3. Add the prepared sample (prepare sample by mixing protein extract with equal Binding buffer) to the column, collect the effluent liquid which can be analyzed by SDS-PAGE.

   Note: For maximal binding, the sample can be incubated for 30 min at room temperature or 4 °C. Be careful not to exceed the resin's binding capacity.
4. Add 2 resin-bed volume Wash buffer to the column to remove the non-specific adsorption protein. Collect the wash liquid which can be analyzed by SDS-PAGE. Repeat this step for six times.
5. Add 5-10 resin-bed volume Elution buffer to the column to wash the target protein, or until the absorbance of the effluent at 280 nm is stable. Collect the wash liquid, and analyzed the content in each tube respectively.
6. Examine and identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or Western blotting.

Storage of the Column

Use 2 resin-bed volume Binding buffer and 2 resin-bed volume deionized water to equilibrate the column in turn, repeat twice. Then add 2 resin-bed volume 20% ethanol, repeat once. Add equal volume PBS containing 20% ethanol as storage buffer, store the column in 4℃ to keep bacteria away.

Cleaning-in-Place (CIP)

In general, resin may be used at least five times. When a column used to purify protein from cell exact usually has buildup of insoluble substances, that is, cell debris, which are non-specifically absorbed onto the matrix support and cannot be completely removed during washing steps. If the column is to be reused, these contaminants should be cleaned from the column. Cleaning-in-Place helps eliminating materials and preventing progressive buildup of contaminants.

1. Add 3 column volumes of deionized water.
2. Add 3 column volumes 0.1% SDS or 0.5 M NaOH solution.

3. Add 3 column volumes of deionized water. Add equal volume PBS containing 20% ethanol as storage buffer, store the column in 4℃.

Trouble Shooting

Problem	Cause	Solution
Back pressure exceeds	Column is clogged	Cleaning-in-place. Increase the centrifugation speed or filtering the sample.
Sample contamination	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply larger sample volume.
Low protein/sample recovery	Target protein is degraded	Perform purification at 4 ℃ in the presence of protease of protease inhibitors.
	Lots of amylase exist in sample or buffer	Add glucose in culture medium to inhibit amylase expression.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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