Lifetech P Series Biotin-Tag Streptavidin Resin 6FF (1ml x 10)

INSTRUCTION MANUAL

FOR KIT No: LT60302RMBKBA

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MANUAL VERSION 1.02

ASSAY PROTOCOL

Reagent Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers by passing through a 0.22µm or 0.45µm filter before use. For most proteins, the following buffer are recommended

Binding of biotin or biotinylated substances:

Binding /Wash Buffer: 20mM NaH2PO4, 0.15M NaCl, pH7.4 Elution Buffer: 8M Guanidine-HCl, pH1.5

Purification of iminobiotinylated substances:

Binding/Wash Buffer: 50mM (NH4)2CO3, 0.5M NaCl, pH10.0 Elution Buffer: 50mM NH4Ac, 0.5 M NaCl, pH 4.0

Sample Preparation

The sample should be centrifuged and/or filtered through a 0.22µm 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. Avoid using protease inhibitors or other additives that contain chelators, such as EDTA, or strong reducing agents, such as DTT or β-mercaptoethanol, which will disrupt the function of the resin. Be careful not to exceed the resin's binding capacity.

ASSAY PROCEDURE

Protocol 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 minutes 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**.**

After-use Storage

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 1xPBS containing 20% ethanol as storage buffer, store the column in 4°C 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 extract usually has buildup of insoluble substances and 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.

To remove precipitated or denatured substances: Wash with 2 column volumes of 6M guanidine hydrochloride, immediately followed by 5 column volumes of PBS, pH 7.4.

To remove hydrophobically bound substances: Wash with 3~4 column volumes of 70% ethanol or 2 column volumes of 1% Triton™ X-100, immediately followed by 5 column volumes of PBS, pH 7.4.

VALIDITY & STORAGE: at per label on box.