MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

MCE MedChemExpress ®

# Strep-Tag Ⅱ Agarose

## 1 Packing list

| Components | HY-K0239-5 mL | HY-K0239-10 mL | HY-K0239-50 mL |
|---|---|---|---|
| Strep-Tag Ⅱ Agarose | 5 mL | 10 mL | 10 mL × 5 |

## 2 Introduction

Strep-Tag Ⅱ tag is composed of 8 amino acids (WSHPQFEK).
MCE Strep-Tag Agarose is a highly cross-linked 4% agarose gel, synthesized through the chemically directed, high-density attachment of streptactin XT protein, can be used for the detection and purification of Strep-tag II and Twin Strep-tag II proteins from various expression sources such as E.coli, yeast, insect cells, and mammalian cells.

## 3 Characteristics

| | |
|---|---|
| Composition | 4% cross-linked agarose |
| Ligand | Streptactin XT protein |
| Binding Capacity | 10 mg Twin Strep-tag II protein/mL |
| Bead Diameter | 45-135 μm |
| Pressure | 0.3 MPa, 3 bar |
| Flow Rate | 80-150 cm/h |
| Gel Concentration | 50% slurry |
| Storage Solution | 1× PBS containing 20% ethanol |

## 4 General Protocol

### Buffer Preparation

| | |
|---|---|
| Binding/Washing Buffer | 20 mM $NaH_2PO_4$, 150 mM NaCl, pH 7.2 |
| Elution Buffer | 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1-5 mM D- Biotin, pH 7.2 |
| Regeneration Buffer | 10 mM NaOH |
| Storage Buffer | 1× PBS, 20% ethanol |

Note: a. It is recommended to prepare all buffers with ultrapure water and. After preparation, filter them through a 0.45 μm or 0.22 μm membrane for sterilization.
b. D-Biotin tightly binds to Streptactin, the binding capacity of agarose will decrease after regeneration with regeneration buffer.
c. The above buffer formulation is for reference only and can be adjusted according to the experiment.

## Protocol

It is recommended to filter the sample with a 0.22 μm or 0.45 μm filter before purification.

### Medium-pressure chromatography

1. Load the column: Load Strep-Tag II Agarose in an appropriate chromatography column and connect it to the chromatography system.
2. Equilibration: Equilibrate the column by washing it with 5× column volumes of Equilibration Buffer. Repeat 2-3 times.
3. Loading: Loading sample using a pump or sample loops, collecting the effluent. Repeating this loading process can improve the binding efficiency.

Note: a. Please choose a suitable gel volume according to the amount of protein to avoid exceeding the loading capacity of agarose.
b. An increase in sample viscosity or volume may lead to column backpressure.

4. Equilibration: Wash the column with approximately 5-10× column volumes of Equilibration Buffer to equilibrate the column.
5. Washing: Wash the column with approximately 10-20× column volumes of Washing Buffer to remove nonspecifically adsorbed hetero-proteins and collect the wash effluent until the absorbance at 280 nm stabilizes.
6. Elution: Elute the column with approximately 5-10× column volumes of Elution Buffer, and collect the eluate in separate tubes based on the absorbance at 280 nm.
7. The eluted protein should be dialyzed into an appropriate storage solution (such as 1× PBS), according to the requirements of the experiment.

### Gravity column method

1. Load the column: Choose an appropriate volume of Strep-Tag II Agarose suspension according to the amount of samples and load it into the gravity chromatography column, eliminating the protective solution.
2. Equilibration: Equilibrate the column: Equilibrate the resin with 5× column volume of Binding Buffer, repeat 2-3 times.
3. Loading: Loading sample and collect the effluent. It is recommended that the samples maintain contact with the agarose at least 2 min.
   Note: Repeated loading can improve the binding efficiency.
4. Washing: Wash resin with 10-15× column volume of Washing Buffer to eliminate non-specifically adsorbed hetero-proteins, collect the effluent.
5. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, collect the eluent, the collected eluates contain the target protein.
6. The eluted protein should be dialyzed into an appropriate storage solution (such as 1× PBS), according to the requirements of the experiment.

### Centrifugation

1. Preparation of Agarose: Choose an appropriate volume of Strep-Tag II Agarose suspension according to the amount of samples and transfer it into a tube, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Wash the resin with 5× the volume of Washing Buffer, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Repeat 2-3 times.
2. Binding: Add the sample and incubate at 4°C for 2-4 h or overnight.
3. Washing: Centrifuge at 1,000 rpm for 1 min and discard the supernatant (e.g., if required, save supernatant for subsequent analysis). Wash the resin with 5× the volume of Washing Buffer, centrifuge at 1,000 rpm for 1 min and discard the supernatant. Repeat 3-5 times.
4. Elution: Elute the resin with 3-5× the volume of Elution Buffer, incubate at room temperature for 5-10 min. Centrifuge at 1,000 rpm for 1 min and collect the supernatant. Repeat 2-3 times and collect the supernatant each time.

### Regeneration

1. 5× column volumes of deionized water.
2. 10× column volumes of Regeneration Buffer.
3. 10× column volumes of deionized water.
4. 5× column volumes of Storage Buffer, store at 2-8°C.

## 5 Storage

4°C, 2 years

Do not freeze

## 6 Precautions

1. Please resuspend the gel thoroughly before use.
2. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.